A History of Anticoagulants

A Symposium

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:OVERY AND EARLY DEVELOPMENT OF ᐊGULANTS: A HISTORICAL SYMPOSIUM

Guest Editor: IRVING S WRIGHT, M.D

Introduction

By IRVING S. WRIGHT, M D

"AT EVERY CROSSWAY on the road that leads to the future, each progressive spirit is opposed by a thousand men appointed to guard the past. Let us have no fear lest the fair towers of former days be sufficiently defended The least that the most timid among us can do is not to add to the immense dead weight which nature drags along

Let us not say to ourselves that the best truth always lies in moderation, in the decent average This would perhaps be so if the majority of men did not think on a much lower plane than is needful That is why it behooves others to think and hope on a higher plane than seems reasonable. The average, the decent moderation of today, will be the least human of things tomorrow At the time of the Spanish Inquisition, the opinion of good sense and of the good medium was certainly that people ought not to burn too large a number of heretics, extreme and unreasonable opinion obviously demanded that they should burn none at all

Let us think of the great invisible ship that carries our human destinies upon eternity Like the vessels of our confined oceans, she has her sails and her ballast The fear that she may pitch or roll on leaving the roadstead is no reason for increasing the weight of the ballast by stowing the fair white sails in the depths of the hold. They were not woven to molder side by side with cobblestones in the dark Ballast exists everywhere; all the pebbles of the harbor, all the sand of the beach, will serve for that. But sails are rare and precious things; their place is not in the murk of the well, but amid the light of the tall masts where they will collect the winds of space."

These timeless words are from "Our Social Duty" by Maurice Maeterlinck.

This Symposium has been developed as a tribute to those who did not concern themselves with the past but who pressed forward into the unknown, facing the risks of failure even after years of work, the painful criticism by those defending the past, and, when things go wrong, the self-examination that besets the investigator who works with human subjects

We pay special tribute to Dr. Jay McLean who died on November 14, 1957, after he had come to New York to discuss with me the plans for this Symposium. He was very enthusiastic about the plan of presenting the history of anticoagulants by those who created it History is too often written long years afterwards by others who try to recreate it from masses of papers which are usually incomplete This results in inaccuracies, which are then perpetuated Jay McLean not only made a basic contribution upon which the conception of anticoagulant therapy rests but he did this in 1916 while working as a student in

Professor Howell's laboratory. This should serve as a constant inspiration to successive generations of medical students.

In the selection of the contributors to this symposium, the editor was limited by the space available. While a serious effort was made to include those whose steps most significantly advanced the discovery, development, application, and evaluation of anticoagulants, it is realized that many other workers whose contributions were important regretfully could not be included as authors. References to some of their work may be found in the texts.

The authors were encouraged to write of their experiences in a personal way. Their scientific findings have all been published elsewhere and are readily available, but what impelled them in this direction, what they felt and believed as they moved ahead, this only they can tell—others can never recapture it in the same degree. The versions do not agree in all respects. This was anticipated and no attempt has been made to reconcile the differences. These are honorable men and they have told their story as they saw it. The differences are like those so common in a courtroom—the readers now and in the future will serve as the ultimate jury. It is nevertheless a profound experience to relive this exciting phase of medical history with those who made it.

The Discovery of Heparin

By Jay McLean, M.D.

THE DISCOVERY of heparin came as a result of my determination to accomplish something by my own ability. Just when this motivation arose in me and what factors nurtured this determination, which was not necessarily fully developed before I went to Johns Hopkins, are difficult to date. I believe the mile posts were the death of my father, John T. McLean, M.D., when I was 4 years old, the remarriage of my mother when I was 9, the earthquake fire in San Francisco when I was 15, the letter of my stepfather when I was 22 discontinuing any further support of my studies in 1922, and a talk with my cousin, Herbert McLean Evans (my father's sister's son), on academic behavior as a student at Johns Hopkins.

I was reared without a father, and a child knows when there is no breadwinner to rely upon My stepfather was unsympathetic to my plans for a medical education at Johns Hopkins The earthquake and fire in San Francisco in 1906 stripped us of all accumulated assets; our house burned, my stepfather's place of employment burned, and the outlook was stark.

Despite these handicaps, I made the decision to become a physician during my last year at Lowell High School in San Francisco (1908-1909). At this time I read Flexner's *Medical Education in the United States.* I have remembered he described the "chem lab" of one school as "consisting of a cigar box of broken test tubes." I entered the University of California at Berkeley (1909), and while there firmly hitched my future to Johns Hopkins Medical School and a career in academic surgery

At that time (1911) one could enter the University of California Medical School with two years of college preparation, but Johns Hopkins required at least three I was forced at the end of my sophomore year, May 1911, to make the choice. My stepfather argued that the University of California Medical School had sufficed for my father (M.D. 1867) and for his 15 year old brother, Robert Armistead McLean (M.D. 1876), Professor of Surgery and Dean, Emeritus, in 1911. After his death in 1918, he was honored in the medical literature as "California's First Master Surgeon."

My argument was that Johns Hopkins offered me more preparation in the field of academic surgery, that is, research and teaching, for a lifetime career Also I felt deeply the responsibility of being a physician. I doubted if I possessed the qualifications to become one; and I deliberately chose the fiercest student competition, as Johns Hopkins' matriculants were meticulously chosen.

My stepfather had paid for my board and room, \$27.50, during my freshman and sophomore years at college; the rest I earned. He offered to continue this for four years of medical school at the University of California in San Francisco If I had decided to go to Johns Hopkins, his aid would be stopped at the end of my sophomore year, May 1911.

Summer work would not yield enough in savings to finance my third college year, so I was forced to leave college for fifteen months. Some of my Sigma Chi Fraternity brothers were going to the Mojave Desert gold mine, The Yellow Aster, at Randsburg, for practical experience in mining engineering. I followed them—got a job as "mucker" (twenty-five cents an hour)—rose to chuck-tender, apprentice miner, and then to a mill-hand, where we processed the ore into beautiful gold bricks. I stayed there until August 1912, fifteen months in all, with enough money saved to re-enter college then for the third year of preparation for Johns Hopkins My spare time was devoted to various part-time jobs. Robert Sproul, now President of the University of California, and I both worked in the Recorder's office. I did blood counts and urinalyses in the College Infirmary, worked in the Museum of Invertebrate Zoology, and book-stores. I also worked scrubbing the decks of ferry boats plying in San Francisco Bay

lumberyards, and as Railway Mail Clerk from Oakland to Denver. All of this was not new to me as I worked at various odd jobs since the age of twelve

In the fall of 1913 I commenced my final year at the University of California which was concurrently my first year as a medical student, and was graduated May 1914 with a B S. degree—but "broke" again. My ill uncle, Robert Armistead McLean, sat with the faculty on the stage of the Hearst Greek Theater. One of my subjects in the first year of medicine was physiology under Professor Maxwell. Howell's textbook was used I was fascinated with the subject and its research possibilities. I wanted to do some research there; but all my spare time was devoted to my job as a technician in the clinical pathology laboratory at the College Infirmary.

I applied for admission to Johns Hopkins but later learned that my dean at the University of California had written the dean at Johns Hopkins that "I was not the kind of man Hopkins sought " In addition, I had no money for the transcontinental journey, let alone the expenses for an academic year at Johns Hopkins

So I returned to remunerative labor, this time drilling oil wells. Manual labor paid so much more than "white collar jobs" and living costs were lower—hence producing greater savings for my purpose.

Again, after fifteen months of work, I had funds for one year at medical school. Even though I had been notified I was not acceptable as a student at Johns Hopkins, I bought a ticket from San Francisco to Baltimore and went there after paying off a senior class loan to the University of California.

I arrived in Baltimore one Sunday morning at Port Royall Station and trudged with my suitcase to the Washington Monument, the first to be erected to him in the United States, and to the Stafford Hotel nearby My object in going to Baltimore, knowing that I had been rejected for admission to the second year class was twofold. I reasoned that I could work a year there as well as in California; secondly, after my 1914 graduation from the University of California, Johns Hopkins had added organic chemistry lab to lectures as a requirement for admission. Working in the oil fields, I could not acquire this subject. I calculated I could work in Baltimore and make this up at Johns Hopkins University at Homewood.

Monday, the next day, I went over to Johns Hopkins Medical School and Hospital and introduced myself to Mr. Coy, the Registrar, and to Dr. W. Williams, the Dean. Then I arranged to share a room on Biddle Street with Irwin Schumacher, now on the faculty of the University of California in San Francisco Arnold Rich, now Professor of Pathology at Johns Hopkins, and James Cash, now Professor of Pathology, University of Virginia, were roommates next door. Mr. Coy was surprised to see me and asked if I had not received the letter denying me admission. I told him I had, but figured on working a year; and I started to look for a job. The next day word was sent to me to see the Dean. I was informed there was an unexpected vacancy and I had been admitted to the school in the second year of medicine.

I promptly paid the fees for a year as a medical student, taking no medical school courses. I immediately called on Dr. Howell and told him of my desire to prepare for an academic career in surgery and that I wished to devote one whole year to physiological research now. I felt that I could never do it after graduation for that would interfere with the house officer progress on a surgical staff. I told him then that I wanted a problem I could reasonably hope to finish and publish in one academic year entirely by myself. I wanted to determine if I could solve a problem by myself. I told him my savings would just last one year, and after that I would have to work a year before returning to school.

He gave me the problem of determining the value of the thromboplastic substance of the body. He thought this to be kephalin (cephalin), obtained from brain but, of course, knew the thromboplastic material from brain to be a mixture—a crude extract, though a powerful thromboplastic agent. He made this by macerating brain tissue, spreading it on glass panes, drying it over a gas flame in an oven, extract-

ing it in ether, decanting, concentrating the ether extract, and finally by precipitation with alcohol This precipitate was his thromboplastic substance He used it in blood-clotting experiments. It was kept in a glass vessel with ground glass cover (vaselined), as it was observed that access of air decreased its ability to accelerate clotting. In three months it was decayed

My problem was to determine what portion of this crude extract was the active accelerator of the clotting process and to that end, to prepare cephalin as pure as possible and determine if it had thromboplastic action I was also to test the other components of the crude ether-alcohol extract I was assigned a sink and attached "table-drainboard" with a shelf over the sink in a large student physiology laboratory (not used as such then) across the hall from Dr. Howell's office and private laboratory.

Others working in the department at the time (1915) were Charles Snyder, Donald Hooker, Cecil and Mrs Drinker, and Stanley Cobb I was held distantly by them, except by Dr. Snyder. They, with Dr. Howell, lunched together, but I was not invited to join them. I was not a colleague This may also have been in part because my drying tissues produced an all-pervading insufferable odor which penetrated throughout the laboratories on the floor and to Dr Abel's laboratory on the floor above!

At the same time I started the organic chemistry laboratory course, to wipe out an entrance deficiency, and voluntarily took an advanced course in German, the better to read the German chemistry literature on lipoids Hugh MacLean's book, however, was in English.

It was this determination to become a physiology-based surgeon rather than an anatomy-based surgeon that led to the discovery of heparin In those days, 1912-1913, anatomy was considered the main foundation for surgery, as it had been for Robert A McLean and my father

My key decisions were thus as follows:

1 Study medicine
2. Academic career.
3. Johns Hopkins.
4. Physiology.
5. Investigate the brain and other organs for thromboplastic agents
6 Study for deterioration of cephalin.
7. Save these longer in event heparin action came up (dog experiment).

In 1915 my cousin, Herbert McLean Evans, M.D., Sc.D, moved from Johns Hopkins to the University of California as Professor of Anatomy. I met him for the first time the day before I left for Baltimore. He gave me the following advice: "Ask no questions but look up for yourself what you want to know" He gave me many letters of instruction to his friends, members of the faculty at Johns Hopkins. Except for one to Dr. Howell, I did not present them as I wanted to progress by my own efforts

I worked nights, Saturdays, and Sundays and the first steps of my problem were completed in December 1915 I still had enough money for board and lodging until June 1916 so I could continue to work in research without receiving any stipend from the medical school I suggested to Dr Howell that it might be profitable to extract the lipoids (phosphatides) from many different organs I reasoned that as cephalin could not be crystallized, one could not be sure of its purity and hence, its function as the thromboplastic substance of the body However, if the thromboplastic activity of brain extract were due to some other substance, adherent to or absorbed by cephalin, this might not be so in organs which did not contain such a large amount of cephalin as the brain does

In my reading of the German chemical literature on phosphatides, I found articles by Erlandsen and Baskoff in which they described extracts of heart and liver secured by a process similar to that for obtaining cephalin from brain. Therefore, these products might be heart and liver cephalin, but were named cuorin (from the heart) and heparphosphatide (from the liver) · hence the name heparin I suggested this research problem as a logical supplement to the problem Dr. Howell had given me He had not known about cuorin or heparphosphatide.

I first prepared cuorin. The final extract was brown, not white or yellow like cephalin. It was waxy. It was a powder. It did not smell "fishy" as does cephalin and although it accelerated the clotting of blood somewhat, it was not as powerful as brain cephalin.

I then prepared Baskoff's heparphosphatide with a similar result. As in the brain, the more "purifications" done (ether extract into hot alcohol), the weaker the thromboplastic activity became. The same process of extraction was used for brain, heart, and liver, yet in the brain, the end product was almost *all* cephalin, but in the heart and especially in the liver it was something else which was mixed with cephalin. As cephalin is powerful, a small amount of it gives ample evidence of its thromboplastic power. Many batches were made of both cuorin and heparphosphatide. By this time, what little cephalin remained from my former studies with brain tissue was deteriorated by the process of extraction plus air and time. I was about to go on to the extraction of cephalin from the uterus and skin. I had saved batches of cuorin and heparphosphatide and from time to time tested these in serum plasma to determine whether or not the cephalin from the heart and liver deteriorated and lost its thromboplastic power as did that from the brain. If I had not saved them, I would probably not have found heparin.

This was a fortuitous decision. All I was trying to prove was that an ether-soluble, alcohol-insoluble extract of cephalin would accelerate coagulation of blood, and it did.

I became interested in the deterioration of cephalin (an unsaturated fatty acid), which I assumed became saturated on exposure to air (and ether-alcohol purification). It seemed sound to determine the iodine number of fresh cephalin in various stages of its decay down to no activity—about 3 months—by exposure to air. This Arbeit was completed and published the following year (1916-1917) at the University of Pennsylvania

The various batches were tested down to the point of no thromboplastic activity, but two of those first prepared appeared not only to have lost their thromboplastic action, but actually to retard slightly the coagulation of the serum-plasma mixture. I had in mind, of course, no thought of an anticoagulant, but the experimental fact was before me; and I retested again and again until I was satisfied that an extract of liver (*more than heart*) possessed a strong anticoagulant action after its contained cephalin had lost its thromboplastic action.

At first I said nothing to Dr. Howell about this. It was not part of my planned *problem*, and it took time to satisfy myself. I had been working alone, in no wise assisting Dr. Howell. He was then engaged much of each day in a dark room watching precipitates of fibrin form through a microscope.

After more tests and the preparation of other batches of heparphosphatide, I went one morning to the door of Dr. Howell's office, and standing there (he was seated at his desk), I said, "Dr. Howell, I have discovered antithrombin." He smiled and said, "Antithrombin is a protein, and you are working with phosphatides. Are you sure that salt is not contaminating your substance?"

I told him I was not sure of that, but it was a powerful anticoagulant. He was most skeptical. So I had the Diener, John Schweinhant, bleed a cat. Into a small beaker full of its blood, I stirred all of a proven batch of heparphosphatides, and I placed this on Dr. Howell's laboratory table and asked him to tell me when it clotted. It never did clot.

He still did not believe that I had discovered a natural anticoagulant, but it was at this point that he became associated in my research problem, namely the study of the effects of my anticoagulating substance (heparphosphatide), which gave greater yield and higher anticoagulating potential than cuorin in vivo in dogs. When I demonstrated new batches to him in vitro, and he became satisfied that it did actually inhibit the coagulation of the serum-plasma test mixture as well as whole blood in vitro, we planned the first in vivo experiment with a dog and *administered* the heparin intravenously.

(This was as far as Dr. McLean progressed in his history of the discovery of heparin *before he* developed his fatal illness and died November 14, 1957.—Ed.)

Preparation of Heparin and Its Use in the First Clinical Cases

By Charles H. Best, C.B.E., M.D., D.Sc., LL.D., F.R.S.

MANY OF US, who were friends of the late Dr. Jay McLean, had looked forward with great pleasure to seeing him again at this time and to discussing the problems which occupied so much of his attention. We all join Dr. Wright in paying tribute to Dr. McLean, the discoverer of heparin, and to Professor W. H. Howell and his colleagues, who extended this work and focused our attention on many of the most important problems in this field. A number of years ago Dr. McLean wrote to me and asked if we would take the responsibility for his collection of notes and reprints and other documents relating to heparin. I was honoured and extremely pleased to accept this invitation.

It is almost always true that a very careful search of the literature will reveal papers which anticipate, to varying degrees, the discovery of a signal advance in medical or other sciences. In 1912, Doyon[1] published a paper in which he describes an attempt to isolate and characterize an anticoagulant released by the injection of peptone in a dog. This work was interrupted by World War I. There are a number of other intriguing findings in the literature, for example that of Schmidt[2] in 1892, but their significance could only be appreciated after the discovery of heparin by Dr. McLean[3] in 1916.

On November 14, 1940, Dr. Jay McLean wrote me a long letter describing the whole history of his work on heparin, and a great deal about his subsequent researches. I will quote parts of this letter.

> You may, however, be interested to know that the first presentation of the anticoagulant at a scientific society was made February 19, 1916, before the Society of the Normal and Pathological Physiology at the University of Pennsylvania. A. N. Richards, the Secretary was then Professor of Pharmacology, and is now Vice-President of the University for the Medical Sciences. These talks were not published although the secretary may have a record in the minutes of the Society. . . . Concerning the lack of articles on heparin in the literature by me, you may be interested in the following. When I wrote the paper on "The thromboplastic action of cephalin," Doctor Howell did not think that I should include anything about the discovery of the anticoagulant. He felt that this should be studied more thoroughly and a paper written about it later. I argued, however, that I had made this finding during that academic year's work in 1915-1916, and felt that it should be included as a record of the work done during that period. I felt this the more strongly because I had already accepted a Fellowship in the Department of Research Medicine at the University of Pennsylvania under Dr. Richard Pearce for the following academic year, 1916-1917 and therefore could not continue the work in Baltimore. He finally agreed to permit its inclusion in the body of the paper.
>
> . . . At first, Doctor Howell was very skeptical that I had found a true anticoagulant. You know that from my method of preparation, I was using very weak heparin and therefore its anticoagulating action was not noticed with the suddenness and brilliancy of an exploding bomb. Furthermore, you will recall that I was searching for coagulants, not an anticoagulant, and that the end point of my experiments was a clot such as is promptly and solidly formed by cephalin. It was only through very careful records, the systematic saving of the little tubes in which I tested the substances, and then repeating the experiments with the same lot of material and finally making new preparations that I gradually became aware that I had an anticoagulant. Naturally I regard the statements in the literature that I discovered this "accidentally" as not correct. It was discovered "incidentally" in the course of the problem but not "accidentally."
>
> . You will find in the beginning of my laboratory note-book, which I am sending you, the extent of the problem Doctor Howell outlined in his own handwriting, namely, "The preparation of pure cephalin." In looking over this note-book, will you tolerantly excuse its lack of neatness?
>
> . . . As regards the earlier studies with the anti-

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coagulant, you might be interested in the following: one author calls my attention to the fourth sentence in the first paragraph of my 1916 paper, which would give one the impression that Doctor Howell suggested that I study cuorin and heparphosphatid for their thromboplastic action. The facts are that the problem Howell originally gave me was simply to make cephalin as pure as possible from the brain and to test each fraction I separated out in the phosphatid group for its thromboplastic action. I finished most of this work between October 15 and January 1916 . . . I first prepared cuorin in January 1916, and it was in January, February and March that I established definitely its anticoagulating action, first of cuorin and then heparphosphatid. It was not until later that Doctor Howell became actively associated in work with the anticoagulant by intravascular injections and mechanism of action in vitro.

I can't think of any other material I have that might be of interest to you. May I, however, offer a suggestion which you may or may not deem worthy of mentioning in your lecture. Doctor Howell has always been perfectly clear and fair in his statements about the discovery of the anticoagulant. As the years go by, more authors credit him with the discovery, apparently disregarding my 1916 publication and the statements he made in his 1917 and 1918 publications. In his Harvey Lecture, he definitely states that this work was done by me, and in his 1918 paper you will note that he says "In the course of his (*that is Jay McLean's*) work, the anticoagulating action was discovered." Doctor Howell has always simply stated that he and Holt "first described" heparin.

Dr. McLean attempted several times to return to active experimental work in the heparin field but was engaged in clinical practice. It was apparent from correspondence which I had with Dr. McLean that he had been trying to interest the United States Public Health Service and institutions in various other countries in doing something about the preservation of his notes, reprints, and other heparin memorabilia. He finally decided to send all of these historical documents to us in the Department of Physiology and the Banting and Best Department of Medical Research. The documents are now stored in the Library of The Charles H. Best Institute.

I had many friendly letters from Dr. McLean. He was most generous in his appreciation of the contribution of our group in Toronto. On May 6, 1940, the discoverer of heparin wrote, "I regard you and the work you stimulated in Toronto to have brought about the debut of heparin for clinical use." My colleagues, Arthur Charles, David Scott, Gordon Murray, Louis Jaques, and T. S. Perrett, deserve a very large share of this praise.

In 1918, Howell and Holt[4] proceeded with the extension of McLean's work. They state, "Attention was first called to this substance during some work done in this laboratory by Jay McLean," that is, to the substance heparin. Howell and Holt go on to say that they varied the methods in many different ways, and finally selected one which yielded a reliable preparation of heparin. In the copies of these articles in the McLean files, there are many interesting marginal comments; for example, Dr. McLean has pointed out that this description of his work by Professor Howell and Dr. Holt was really the first published announcement of the discovery of heparin. There are many interesting points also in Howell and Holt's paper. They introduced, for the first time, the word "heparin"; McLean had referred to these compounds carrying the anticoagulant activity as "phosphatids from heart or liver." They found that heparin could be prepared from lymph glands as well as from heart and liver, as originally shown by McLean. The antagonism between cephalin and heparin on the clotting system was described in the Howell and Holt paper. It has been questioned whether the material that Howell and Holt had was actually heparin, since it was soluble in the crude form in ether. It is now considered that it probably was heparin, since it became insoluble in ether after repeated alcohol precipitations. In 1922 and 1925 Howell[5,6] described the preparation of heparin in more purified form and in 1928 he[7] published a detailed report on its chemical and physiologic reactions.

In 1924 Mason[8] showed that heparin would prevent the intravascular clot produced in rabbits and dogs by the injection of thromboplastin from tissue extracts. These were true clots and not platelet thrombi. In 1925 my close friend C. I. Reed[9] found that heparin was an effective anticoagulant in dogs and

was well tolerated. In 1927, Shionoya[10] reported that the administration of heparin did not prevent the agglutination of platelets when blood was made to pass through a collodion tube. Thus it seemed that heparin might be an anticoagulant but not an antithrombotic agent.

Professor Howell undoubtedly anticipated many of the developments which took place in the future. He expressed the hope that heparin would find a suitable application in experimental work and possibly in the therapeutic treatment of disorders of coagulation. Professor Howell thought it not improbable that this substance might be of physiologic significance, and in discussions on coagulation of the blood he often referred to heparin as a "physiological anticoagulant."

While working in Dale's laboratory in London in 1928 I had decided to organize a group, on my return to Toronto, to study the chemistry and physiology of heparin. Later that year, Dr. E. W. McHenry and I, eager to use an effective anticoagulant in our histaminase work, found it possible to prepare active fractions from ox liver by Howell's method. A little later I made a comprehensive study of the literature and it became apparent that very little work indeed was being done in this field. A potent anticoagulant that could be used for long continued administration in animals, was not available. No anticoagulant preparation was safe for clinical work and none was being used. In the Connaught Laboratories I had been intimately concerned with the preparation of insulin and of liver extract for administration to human patients and I visualized a similar advance in the heparin field. Progress was, apparently, also inhibited by the lack of convincing evidence that heparin inhibited platelet agglutination as well as coagulation.

It was obvious that further chemical work on the purification of heparin must precede physiologic and clinical studies. In 1929 I was able to interest a young organic chemist, Mr. Arthur Charles, in this problem, and he made some preliminary studies with me in the Department of Physiology and then joined forces with my colleague of long standing, Dr. D. A. Scott. From that time on the chemical work on heparin was conducted in the Connaught Laboratories, of which I was then an Assistant Director.

On November 10, 1931, I wrote to Professor W. H. Howell.

> I would very much appreciate your opinion with regard to several questions in connection with heparin. During the last few years we have been using great amounts of this material in physiological and bacteriological work. Quite recently, one of the junior members of the Connaught Laboratories, which, as you know, are a department in the University, has interested himself, at my suggestion, in the preparation of heparin from beef liver. He is now in a position to make fairly large amounts of the material which is at least as potent as that distributed by Hynson, Westcott and Dunning. One half gram of this material is being forwarded to you under separate cover. Would you have any objection if this material should be sold by the Connaught Laboratories? (*Now the Connaught Medical Research Laboratories whose objectives are the support of research by the sale of biological products at the lowest possible price.*) I believe that the price would be much more reasonable. As you know, there is a very high tariff on biological products going into the United States so there is very little likelihood of any interference with the American business of Hynson, Westcott and Dunning.

On November 14, 1931, I received the following reply from Professor W. H. Howell in his own handwriting.

> I am interested and pleased to know that you have got a usable preparation of heparin out of beef liver. I never could make that source give a decent preparation. As to your selling it, there can be no objection to that, of course. I have feared, at times, that the Hynson, Westcott and Dunning firm would give up its production, as they always claimed that it was a losing proposition to them, so it may be well to have another source. I have been very anxious for them to market a purified heparin, potency 1.50 in 100, but the method I gave to them makes their product too expensive, they think.

The work on heparin in the University of Toronto was the product of activity in three departments—Physiology, the Connaught Laboratories and the Department of Surgery.

Dr. David Scott and Dr. Arthur Charles[11-15] were extremely successful in their chemical work during the years 1933 to 1936. The most important and novel steps in the preparation and purification of heparin which they introduced were (1) the finding that autolysis of tissue resulted in a much higher yield of heparin, (2) the discovery that beef lung yielded almost as much heparin as liver—this made it possible to use a much cheaper source of raw material, (3) the finding that the destruction of protein by trypsin in the crude protein-heparin complex, was an extremely important factor in the further purification of the anticoagulant, (4) the preparation of a crystalline material as the barium salt—they found that this purified material was of uniform composition and potency. The Danish workers, Schmitz and Fischer,[16] had isolated in 1933 the anticoagulant material from dog's liver as the brucine salt. Neither the brucine salt nor the barium salt lent itself to any large-scale production. Somewhat later Charles and Scott were able to convert the crystalline barium salt of heparin into the sodium salt.

The labels on the bottles of heparin prepared by Hynson, Westcott, and Dunning from dog's liver by Howell's procedure, stated "1 mg will prevent the coagulation of 5 cc of cat's blood in the cold." Charles and Scott used this preparation as a reference standard and assigned it a potency of 5 units per mg. In terms of this material the potency of the crystalline barium salt was 110 units per mg. but for simplicity in calculation Charles and Scott decided to assign the figure of 100 units per mg. The material provided for the international standard of heparin was the sodium salt prepared from the crystalline barium salt. The potency of the international standard[17] of heparin was defined as 130 units per mg, that is, there are 130 arbitrary units of heparin per mg of the international yardstick. It is calculated that the potency of the international standard is 28 times that of the early Hynson, Westcott, and Dunning preparation. The Connaught Laboratories in Toronto have made two international biological standards—the one for insulin and the one for heparin.

In addition to obtaining heparin in a highly purified form I thought that another point should be settled before the anticoagulant should be submitted for clinical trial. This was the ability or inability of heparin to prevent the agglutination of platelets, which is the first step in the formation of a thrombus as distinguished from a clot.

In 1929, the year after we started our work on heparin, Professor W. E. Gallie, Head of the Department of Surgery in Toronto, nominated Dr. Gordon Murray to collaborate with workers in my department, who were investigating the effects of heparin in the prevention of experimental thrombosis. I was fortunate in having in my department at that time, Dr. T. S. Perrett, a Fellow from the Department of Surgery. I was also fortunate in having a pupil who was taking his doctor's degree in physiology. This student soon became a colleague in the heparin work and a very close friend. He was, as you know, Dr. Louis Jaques, who later became the Head of the Department of Physiology at the University of Saskatchewan and an international authority on many aspects of blood clotting and thrombosis. Dr Jaques, among the other services which he rendered to our department, sent me one of his own pupils, Dr Frank Monkhouse, who received his Ph D. in Physiology from my department in 1952. Dr. Monkhouse is, therefore, my scientific grandson and he, in his turn, has become an authority on different aspects of the great field of blood coagulation and thrombus formation.

The work on the effect of heparin on experimental thrombosis begun in 1929, was pushed forward by Dr. Murray, Dr. Jaques, Dr. Perrett, and myself. We[18] found that the incidence of obstruction of peripheral veins in dogs by thrombi formed as a result of mechanical or chemical injuries to the intimal surfaces of the blood vessels was definitely decreased when solutions of purified heparin were administered before and for long periods following the injury. These results were obtained in studies of some 300 veins. Thrombi were not observed even after very severe

chemical injury while the animal was well heparinized. We found that the intimal surfaces of veins removed from heparinized animals several days after the injection of heparin had been discontinued appeared, on microscopic examination, to have recovered completely from the injury. The microscopic examinations revealed, in some cases, minute masses of platelets, filling small crevices in the intima. Healing was, however, complete as judged by the absence of thrombus formation after discontinuing the anticoagulant.

The experimental evidence of the prevention of thrombus formation initiated by platelet agglutination, was completely satisfactory before attempts were made to apply solutions of purified heparin to clinical problems

At various stages in the purification of heparin attempts had been made to use the material as an anticoagulant in transfusing human patients In 1924 Mason[18] used crude material and obtained reactions which varied from slight chills to severe headache and high fever and nausea In 1928 Howell[7] used somewhat purer heparin and reported a slight reaction in 2 of 10 transfusions carried out on 6 patients. Godlowski[20] in 1933 reported on the use of heparin in human patients, and although he found low levels of toxicity, the preparation he used was extremely crude and of low potency In 1936 Hedenius and Wilander[21] studied coagulation times of healthy human subjects. They found that heparin produced no ill effects when the material was given intravenously This heparin was obtained from Dr Erich Jorpes and was made by the Charles and Scott procedure

The work on heparin in Toronto, begun in 1928, proceeded steadily With each advance in purification we, Murray, Jaques, Perrett and Best, studied the effect on experimental thrombosis and Dr. Gordon Murray made clinical trials beginning in May 1935 When the crystalline sodium salt became available it proved to be safe and effective for the heparinization of patients

On May 8, 1935, Dr Jorpes wrote to me from Stockholm in his own hand

I am sending you a copy of the preliminary report about heparin, and would like to use this opportunity to thank you for all the hospitality shown to me and to Mr. Bjurling during our visit in Toronto in 1929. We have greatly benefited from your experience in the manufacture of insulin.

The heparin work has been a very hard task. For a very long time I believed that my preparations were only impurities as compared with those of Charles and Scott. I greatly admire their working capacity They have opened this field, which before them was quite hopeless.

On May 28, 1935, I answered

I have been interested in some physiological work on heparin recently; as a matter of fact, we have been administering some to human subjects. I hope that we will see you at the Physiological Congress in Russia this summer.

Up to the time of this letter there had been no published reference to the use of highly purified heparin in clinical cases but as Dr. Jorpes has written, the idea of using heparin to prevent the formation of thrombi was in the minds of all who came in close touch with the problem. Its realization merely depended on the availability of a satisfactory preparation of heparin. The clinical problem was attacked in Toronto and in Stockholm, as soon as pure heparin was obtainable The studies by Crafoord[22] and later by other workers in Sweden, were proceeding at the same time as those of Dr Gordon Murray[23] in the Toronto General Hospital The results obtained clearly indicated that certain types of clinical thrombosis could be prevented by the treatment with purified heparin These findings were made possible by the preparation of pure heparin from beef liver or lung. The word pure is used here to indicate a uniform preparation, of standard potency, and free from toxic components rather than in the true chemical sense

Dr. Jorpes and his colleagues have made a very large number of fine contributions to the heparin field The cellular origin of the anticoagulant, the chemistry, the mechanism of action, the clinical use in a great variety of conditions, and many other subjects have been illuminated by the work of this group, which

is well summarized by Dr. Jorpes[24, 25] in his monographs. I have had the pleasure of knowing a number of the Swedish "anticoagulationists" in addition to Dr. Jorpes. Dr. Per Hedenius and the late Dr. Hjalmar Holmgren have been particularly close friends.

Our present knowledge of the chemistry of heparin has been summarized by Dr. Arthur Charles[26] as follows: "Heparin is a complex polysaccharide. The carbohydrate moieties are glucuronic acid and glucosamine which are present in the molecular ratio of 1:1. The carbohydrate is highly sulphated. The amino group is not free and does not appear to be acetylated as in mucoitin or chondroitin sulphate Evidence has been presented which indicates that the nitrogen is sulphated."

The availability of well standardized heparin not only made possible the clinical work but a very great deal of experimental study. Without this potent heparin the exchange transfusion experiments, carried out by Thalhimer, Solandt, and myself,[28] would not have been possible. The dramatic use of the artificial kidney by Kolff and Berk[29, 30] in Holland and by Dr Gordon Murray in Toronto,[31, 32] also depended on purified heparin I will not attempt to make a complete list of the advances which the availability of potent purified heparin has facilitated

There will obviously not be time to follow in detail the many lines of interest which developed in the middle 1930's. Members of our own group were interested in the source of heparin and its appearance in blood in peptone and anaphylactic shock. The work on Witte's peptone goes back to the publication of Schmidt-Mulheim[33] in 1880, when it was shown that injection of the material in dogs produced shock and incoagulability of the blood. In 1909 Biedl and Kraus[34] found that the blood failed to clot in anaphylactic shock Professor Howell[8] in 1925 and Quick[35] in 1935 had obtained anticoagulant preparations from dog's blood after injection of peptone The subject was further advanced by Wilander[36] in 1939, who isolated heparin in amounts sufficient to explain the coagulation deficiency Waters, Markowitz, and Jaques,[37] in our laboratory, showed in 1938, that the incoagulability of the blood, both in peptone shock and anaphylactic shock in dogs, was completely inhibited by protamine. The dramatic neutralization of the effect of heparin by protamine had been shown by Chargaff and Olson[38] in 1937. In 1940 Jaques and Waters[39, 40] isolated a barium salt of pure heparin from the blood of sensitized dogs given serum albumin.

Another point of interest in our laboratory was the enzymatic destruction of heparin by material prepared form rabbit's liver. This was carried out by Jaques[41] in 1940 and he suggested the name "heparinase" for this system. The use of *silicone* in preventing clotting was introduced by Jaques, Fidlar, Feldsted, and Macdonald[42] in my laboratory in 1946. This was a great improvement over vaseline or paraffin, which, of course, had been used ever since the work of Freund[43] in 1888 and of Bordet and Gengou[44] in 1901. A very great many experiments have been facilitated by the use of silicone coating of glass tubes, needles, and other apparatus

The experimental work which D. Y. Solandt, Reginald Nassim, and I did[45] on the prevention of coronary thrombosis and intramural thrombosis in dogs by the administration of heparin, fascinated us until problems of military medicine diverted our attention in 1939. Dr. Solandt and I[46] described a method by which gradual occlusion of coronary arteries by thrombus formation may be produced in experimental animals. The thrombus formation and the resulting cardiac infarction were in a very large part prevented by the administration of adequate amounts of highly purified heparin. In discussing the possible clinical application of our findings I[47] wrote, in 1938, "If the clinical investigation of cardiac cases should be initiated, the necessity for studying very large numbers and of heparinizing only alternate cases is obvious."

In the investigation which Dr. Solandt and I made with Dr Nassim, we evolved a method by which cardiac mural thrombi could be produced in animals. These thrombi were formed very rapidly and there was a very dramatic and extensive fall in blood platelets during this interval The formation of the mural

thrombi could be completely prevented by the administration of adequate amounts of highly purified heparin. We[41] wrote at that time, in 1939, "Since over ten per cent of the deaths associated with coronary thrombosis in man are caused by embolic sequels of mural thrombus formation, a clinical trial of heparin is indicated."

There was an attempt, in Toronto, to apply some of these results, but no comprehensive investigation was found to be possible at that time. In 1948, Wright, Marple, and Beck[44] wrote, "The possibility of preventing the extension of coronary thromboses and the development of mural thrombi in the presence of myocardial infarction by the use of anticoagulants was suggested by Solandt, Nassim and Best in 1938 . . . Their observations were not applied to human beings on any significant scale because of the difficulties and the risk felt to be inherent in the use of heparin clinically"

When purified heparin became available in Toronto requests for this material for experimental and clinical use came from many parts of the world One of the earliest was from Dr Leo Mayer who wrote on December 21, 1938, "Dr. Irving Wright of the New York Post Graduate Hospital has suggested the advisability of using heparin in this case" The patient was Mr Arthur Schulte I remember sending heparin to Dr I S Ravdin of Philadelphia who needed it for the postoperative treatment of a brilliant young doctor who had a saddle embolus at the bifurcation of his aorta Many surgeons and physicians came to Toronto to discuss the clinical problems with Dr Murray, or physiologic or chemical matters with our group I remember many of these men vividly—Dr Essex and Dr Priestley of the Mayo Clinic and Dr Lahey of Boston were among those who came from this country

The interest in heparin continues to grow. Dr Jay McLean was undoubtedly fascinated by the effect of heparin on the clearing of lipemic plasma, first demonstrated by Hahn,[45] and by the great volume of recent literature on the effects of the anticoagulant on fat mobilization

Heparin has thus already removed many barriers to the free flow of knowledge but we are still in the early stages of appreciation of its physiologic and clinical significance

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Heparin: A Mucopolysaccharide and an Active Antithrombotic Drug

By J. Erik Jorpes, M.D

HEPARIN, like so many other biological substances, was discovered incidentally. William H. Howell, professor of physiology at Johns Hopkins University, Baltimore, was in 1916 trying to isolate a thromboplastin, an accelerator of the coagulation of the blood, from the phosphatide fraction of the liver and the heart. His co-worker, Jay McLean, found instead a substance, later called heparin, which retarded the coagulation of blood. Also unexpected was the finding made almost 20 years later in 1935 that heparin is a mucopolysaccharide esterified with sulfuric acid to a quite extraordinary degree.

The Chemical Nature of Heparin

The closest chemical neighbor of heparin, chondroitin sulfuric acid, was in the 1920's in spite of its interesting chemical composition still not easily accessible. It shared with the nucleic acids the property of being a macromolecular ester of a strong mineral acid, in this case sulfuric acid. In 1928, I succeeded in obtaining a protein-free chondroitin sulfuric acid having an almost theoretical content of ester sulfate by adsorbing the proteins on kaolin. This preparation was then used in our laboratory as a reference substance for checking the methods used for the quantitative analysis of uronic acids.

Among other natural products analyzed for uronic acids we also included in 1934 the heparin, claimed by Howell to give a positive color reaction for uronic acid. Heparin had been isolated in 1933 in a highly purified state by Charles and Scott of Toronto. In fact, the Tollens-Lefèvre technic showed a considerable content of uronic acid in heparin, a content which increased with increasing anticoagulant activity, making up almost 20 per cent of the dry substance of the purest heparin preparations.

The Tollens-Lefèvre technic is quite reliable and is easy to perform. The same could not be said about the methods applied for the biological assay of heparin. Cats were not so easy to get, and it was difficult to find the operating room and the assistants needed. An easier solution was then found. Series of test tubes containing a glass bead and serial dilutions of heparin solutions were filled early in the morning with fresh ox blood at the slaughter house, and readings of the coagulation times were made at intervals during the day. In 1 day several heparin samples of unknown strength could be compared with a standard heparin practically without any cost. This technic opened the field for further experimentation on a larger scale.

The purified heparin samples were found to contain large quantities of a hexosamine, which was later shown to be glucosamine, amounting to one mole of hexosamine per mole of uronic acid. At that time, 1933, Elson and Morgan had improved the Zuckerkandl-Klebermass method of 1931 for the quantitative analysis of hexosamines. We also believed that we had found acetic acid as a third component like that of the chondroitin sulfuric acid, but this assumption, based on faulty technic, soon proved to be erroneous.

The organic skeleton of heparin thus showed similarity to that of the chondroitin sulfuric acid. This acid, however, has no anticoagulant activity. The analysis of the ash, which amounted to not less than 25 to 40 per cent of the different preparations of the purified heparin, then gave the key to the problem. It was found to consist of sulfates exclusively. The sulfate was precipitable with barium chloride after the heparin was first hydrolyzed with mineral acid. This indicated an ester linkage

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of the sulfates similar to that in the chondroitin sulfuric acid. Now, when the sulfur analysis indicated a sulfate content of about 40 per cent in the purified heparin, 2½ times more than in chondroitin sulfuric acid, difficulties arose in convincing workers in this field that such compounds could exist in nature. It was an easy matter, however, to induce anticoagulant activity in ordinary polysaccharides by treating them with chlorosulfonic acid.

At almost the same time, 1938, however, Soda and Egami in Japan found in the visceral hump of a mollusca, *Charonia lampas*, a similar compound, a polysaccharide containing 15 per cent of sulfur as ester sulfate. Many years later, 1947 to 1950, Vasseur in Sweden showed that the mucous layer surrounding the sea urchin eggs contains plenty of polysaccharide polysulfuric esters with an ester sulfate content of about the same order as that of heparin. The organic skeleton of these compounds is built up of hexoses and methylpentoses, varying for different species

It was thus evident that heparin belonged to a group of natural substances called mucopolysaccharides containing a hexosamine and a uronic acid, and that polysaccharides likewise highly esterified with sulfuric acid can be synthesized even by invertebrate animals One detail, however, in the chemical structure of heparin is unique, the sulfamide or amidosulfuric acid group In heparin, 1 sulfuric acid group is linked to the amino group of the glucosamine, as suggested by Masamune in Japan in 1940 and by Wolfrom in the U S A in 1943 and finally demonstrated by our group in 1949.

It may not be quite out of place to point out that a neglected elementary analysis, in this case the sulfur analysis, evidently delayed the elucidation of the chemical nature of heparin, a by no means uncommon fatality Liebig himself missed the phosphoric acid in his inosinic acid, the first nucleic acid described. Fifty years later it was shown to be a nucleotide The sulfur content of taurine was likewise overlooked by Pelouze and Dumas in 1838 Also the sulfur of the thio-methyl pentose of the yeast was missed, and made the sugar difficult to identify until the sulfur was found by Zuzuki and Mori in 1926. Two oxygen atoms put into the place of 1 sulfur atom made the elementary analysis fit. Even the sulfur of vitamin B_1 escaped detection when the newly crystallized vitamin was analyzed by Jensen in 1926.

It must be pointed out however that Howell, although being a physiologist, did not miss the sulfur in the ash after igniting the heparin preparations, but he found it quite natural to speak about the ash as a contamination of the samples.

Heparin and the Mast Cells of Ehrlich

In 1936 the Stockholm group found that the heparin is produced by the mast cells of Ehrlich, a discovery which gave rise to an overwhelming literature dealing with these cells Lison's finding of 1933 that the purple metachromatic staining of cartilage and mucous membranes given by toluidine blue is due to the ester sulfate group of chondroitin sulfuric acid caused me to apply the reaction to heparin. With heparin it was 100 times stronger than with the chondroitin sulfuric acid.

A stimulating observation was made when swine thoracic aorta was immersed for a short while in a 0 01 pro mille solution of toluidine blue. The most brilliant color in purple lila was developed on the inside of the aortic intima We immediately anticipated that the heparin should form a superficial layer inside the aortic wall which with its ionized ester sulfate groups could exert some kind of repellent action Unfortunately for the hypothesis, the metachromatic staining was due to the chondroitin sulfuric acid present in the intima

The location of the heparin in the mast cells of Ehrlich was made by Hjalmar Holmgren, assistant at the Histology Department of our Institute When we asked him to locate the heparin in the body by means of the metachromatic reaction, he could the next morning inform us that the mast cells of Ehrlich, a kind of cells quite foreign to us at the Chemistry Department, were carrying the heparin

in their granules. The quantitative analysis performed by Wilander also showed 10 times more heparin in the capsula Glissoni, the liver capsule, which is extremely rich in mast cells, than in the liver parenchyma itself. Since that time much, possibly too much, has been written about these cells and many more functions have been assigned to them than the order of Nature reasonably can have bestowed upon them

Heparin as an Antithrombotic Drug

Clarence Crafoord, the well-known Swedish thoracic surgeon, had drawn attention to himself already as a very young physician through his numerous pulmonary embolectomies (between 20 and 30) What would be more natural in a case like his than to go to a biochemist and ask him to get out the heparin of Howell to be tried as a prophylactic against pulmonary embolism This was in fact what Crafoord did in 1929. The only answer we could give him was unfortunately, "*Non possumus*"

In 1935 we instead could approach him at the Sabbatsberg Hospital and ask him to try out our heparin preparations clinically. In the meantime Hedenius and Wilander had performed the first intravenous heparinization on themselves outside of the hospital Their finding that 100 mg. or more of heparin are needed for heparinizing a human being for a few hours caused at first an almost desperate feeling It seemed to be impossible to get sufficient material for a heparinization on a large scale All the work on the chemistry of heparin had been performed on 6 Gm We could not anticipate at that time that we in cooperation with a pharmaceutical house, Vitrum AB, Stockholm, within a few years should be able to produce 1 Kg or more a week of the new substance

Crafoord immediately started a series of experiments heparinizing patients postoperatively Many an older colleague shook his head and expressed his dislike of such experiments, in which the patients were "made hemophiliacs" for a time Crafoord, anyhow, fulfilled his intentions and treated 325 patients with heparin postoperatively His colleague, Per Wetterdal, of the Gynecology Department of the same hospital, contributed another 231 cases and Leissner of the Maternity Clinic of the University of Lund heparinized 309 patients post partum. In total, about 800 cases were thus given heparin after operation or childbirth A high frequency of thrombosis, at least 3 to 4 per cent and possibly still higher, was expected in Crafoord's series if untreated consisting only of patients over 35 to 40 years of age and with operations known to be followed by a relatively high percentage of thromboembolic complications. Practically no incident of that kind occurred. Although Wetterdal's and Leissner's series comprised only selected cases expected to give a high frequency of thromboembolism, no complications were observed during the first 10 to 15 days after operation or delivery. Among the 657 (325 + 140 + 192) cases receiving 250 mg or more of heparin daily for 5 to 10 days no signs of thrombosis occurred.

Similar experiments were at the same time going on in Toronto, Canada In order to demonstrate the usefulness of heparin in inhibiting thrombosis a series of animal experiments, initiated in 1932 in the Department of Surgery of the Toronto General Hospital, was performed in close conjunction with the chemical work on heparin at the Connaught Laboratories of the University of Toronto These experiments were reported by Murray, Jaques, Perrett, and Best in 1936 and 1937. In 1938 Solandt and Best published their well-known paper about the dissolution of fresh thrombi in the coronary arteries of dogs by perfusing the vessels with a dilute heparin solution Gordon Murray at the Toronto General Hospital contributed at first 260 cases and then a total of 400 cases treated prophylactically with heparin He reported results equally as good as the Swedish group

Thus the postoperative course of the more than 500 carefully controlled cases of Crafoord and of Wetterdal, supplemented by the 309 cases of Leissner and the 260 cases of Murray and MacKenzie, a total of 1,151 patients, seemed to prove that heparin, if rou-

tinely used over a sufficient length of time, gives an almost complete protection against thromboembolic complications after surgical operations and childbirth.

Anticoagulant Therapy in Thrombosis

As a result of the lively interest in heparin following Crafoord's first publication in the spring of 1937 on prophylactic heparin treatment in man and our discovery of the connection between heparin and the mast cells of the same year, a physician in Stockholm, Holmin, later in the year tried the new remedy in a case of fresh acute thrombosis in the central retinal vein in a young person. Well aware of the hopeless prognosis, he gave a tentative dose of pure heparin intravenously 3 to 4 times daily over a period of 10 days beginning on the third day of the illness.

Ploman describes the course of this case as quite unusual, for the patient regained a visual acuity of 0.9 in 9 days. In a second case, described by Boström and William-Olsson, where the lesion was 1 month old, visual acuity rose from 0.1 to 0.4 in 5 days and later to 0.6 The unusual course of these 2 consecutive cases made these ophthalmologists inclined to ascribe the result to the treatment with heparin.

In the same year Magnusson (1938) used heparin successfully in a case of thrombosis the posterior inferior cerebellar artery, Wallenberg's syndrome, a disease in which a regression is unusual. In 1938, Murray and Best reported 28 cases of spontaneous thrombophlebitis and 7 cases of pulmonary embolism treated with heparin. All the cases of embolism showed rapid clinical improvement, and the 28 cases of spontaneous thrombophlebitis showed no evidence of embolism, and the clinical signs and symptoms, pain, swelling, tenderness and fever, appeared to show more rapid improvement than in a control group

In his second paper Crafoord (1939) stated that he had given heparin to 20 patients with manifest, thromboembolic complications In some of these both the general and local symptoms receded strikingly rapidly

In Sweden, Magnusson (1940) administered heparin to a woman with severe pulmonary embolism and thrombosis in both legs, complicating a postpartum scarlatina. The temperature became normal in a few days and the patient, who had been ill for 6 weeks with repeated thromboembolic recurrences and was very emaciated, recovered.

After these most dramatic and very convincing preliminary experiences with heparin discussion of the anticoagulant therapy was broadened and taken up on a larger scale in different parts of the world. It was quite evident that there could be no question about a general prophylactic heparinization. Early rising after operations and childbirth makes such a measure superfluous except in some cases with a pronounced tendency to thrombosis. Then Karl Paul Link's work, leading to the discovery and synthesis of Dicumarol, broadened the field in a highly desirable way by making a prolonged anticoagulant therapy possible. The easily accessible oral drug also proved a prerequisite for long-term prophylactic treatment.

It may be added here that active movements and early rising from the bed are now generally prescribed and strictly applied in most countries. The question then arises to what extent the beneficial effects of the anticoagulant therapy observed are due to the anticoagulants or to the movement therapy. In fact the most critical observers in Denmark, the country of Hans Christian Andersen, have spoken about the Emperor's New Clothes in thinking of the advocates of the anticoagulant therapy, a very sound criticism indeed. Without active movements and early rising from the bed the effect of the anticoagulant treatment would certainly not have been so good.

It is in fact impossible to evaluate these experiences correctly. The fact remains, however, that thrombosis can be prevented through prophylactic heparinization. As to anticoagulant therapy in thrombosis, it must also be kept in mind that prior to this therapy thrombosis of the veins of the legs used to be so painful and the legs so swollen that there could be no thought of active movements and early rising Anybody treating a severe leg

thrombosis or a pulmonary embolism with heparin will soon be impressed by the amelioration of the pain and of the feeling of oppression, and by the disappearance of the swelling of the leg. It would also have been deplorable if the medical profession had been unable to detect the value of the movement therapy without the stimulant of the new anticoagulant therapy. Until the most recent years thromboembolic patients were kept in bed for 6 to 8 weeks.

Under the influence of this discussion a gynecologist at one of our university clinics decided to treat a series of cases of acute thrombosis without heparin. The first patient was sent home and instructed to move around as much as possible. A few days later she came back with a florid phlegmasia alba dolens. His series comprised only this case.

In the beginning of the 1940's the large-scale clinical experiments with the anticoagulant therapy had begun in Sweden (Hellsten, Bauer, Zilliacus), in the U.S.A. (Allen, E. V. and Barker, N. W., Wright, I. and co-workers, de Takats, G.), and in Switzerland (Merz, R. W.). Heparin and Dicumarol thereby left their cradle, the laboratories of physiologic and organic chemistry. They both proved valuable enough to keep their position in a world where new therapeutic products flourish and disappear in a continuous stream. An almost immense literature already tells their story.

In speaking about the cradle of heparin it is not out of place to mention that the writer had the pleasure of being able to inform William H. Howell of Johns Hopkins University during his last years about the successful progress of the anticoagulant therapy in this part of the world. Needless to say, these reports were welcome. They told him that something of permanent value will remain as a result of his contributions to physiology. They might also to some extent have enlightened those dark days during the war, as that of April 11, 1942, when he wrote, "When this killing and shooting is all over I fear that this world will not be such a pleasant place to live in as it was in my youth—and I shall have no great regrets in leaving it, although I would dearly love to know what steps will be taken to assure a permanent peace."

The Development and Use of the Prothrombin Tests

By Armand J. Quick, M.D., Ph.D.

Prologue

Take from the altar of the past,
the fire—not the ashes.
Jean Jaures

At the end of the last century, the fire on the altar of coagulation was burning briskly, for it had been well fed with the fuel of ideas and experimental findings by such workers as Buchanan, Schmidt, Hammarsten, Pekelharing, Arthus and Pagès, and Morawitz. After 1900 the flames began to die down, partly because fewer new ideas were forthcoming and partly because the fire was smothered by confused theories and poorly executed experimental work. Such simple instruments of precision as the pipet and the stopwatch had not generally found their way into the physiologic and clinical laboratory; the workers were content to measure volume in drops and to record reaction times in minutes and hours instead of seconds Fortunately, there remained in the embers much that could be rekindled. In 1890 Arthus and Pagès[1] had discovered that the blood became incoagulable when sodium oxalate was added and that the addition of calcium restored clotting. Twenty years later Addis[2] employed the principle of timing the clotting of recalcified plasma, and shortly thereafter Howell[3] standardized the method and demonstrated its usefulness He believed that the test was a measure of prothrombin Since the clotting time of recalcified plasma is prolonged in hemophilia, he concluded that a prothrombin defect accounted for the abnormal coagulation in this disease Later, however, he and Cekada[4] obtained evidence that the prothrombin was normal in hemophilia This conflict made the interpretation of the test difficult and resulted in considerable confusion because the procedure continued to be called a prothrombin test In 1929 Bancroft, Kugelmass, and Stanley-Brown[5] employed the test to study thrombosis and certain bleeding states They observed a delayed clotting in hemophilia and also in a number of cases of jaundice. Nygaard[6] at the Mayo Clinic confirmed the delayed clotting of recalcified plasma in jaundiced patients. He concluded that the coagulability was decreased, but he refrained from offering any explicit explanation. This is understandable when one recalls that at this time the classical theory, which eventually furnished a satisfactory and practical answer, had won little recognition in America.

Act I, Scene I

In 1932 the stage was set for a new attack on the problem of the coagulation of the blood and on the hemorrhagic diseases One scene was at the State University of Iowa in the laboratory of the Pathology Department, the other in a little corner in the laboratory of the Fifth Avenue Hospital of New York Since I played an active role in the latter scene, it is easiest to tell the story in the first person

As already mentioned, Dr. Bancroft and Dr Stanley-Brown had begun a study on the coagulation of the blood with the aim of attacking the problem of postoperative thrombosis. They had obtained a grant of money from Mrs. Blossom, of Cleveland, for this investigation which enabled them to pay a full-time fellow I learned of this through my teacher and friend, the late Dr. Joshua Sweet, and when I was offered the opportunity to work with them, I accepted with some hesitation and trepidation for I knew nothing about blood coagulation.

From Mrs Charlotte Breitung, the technician, I learned the technic of the clotting time of recalcified plasma. As the studies progressed, I confirmed what Dr. Bancroft and his associates had already reported,

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namely, the prolonged clotting time in hemophilia and in certain patients with obstructive jaundice Difficulty was encountered, however, when I attempted to correlate a thrombotic tendency with a shortening of the clotting time of recalcified plasma It became obvious that the test had to be rigidly standardized To do this I had to know more about the theory of blood clotting and also to gain additional practical experience

In meeting the first requirement, I had the good fortune to have the Academy of Medicine with its excellent library within walking distance. Evening after evening I struggled to find my way through the maze of conflicting theories. To say that the current literature of that period was bewildering is to understate the facts A few investigators denied even the existence of prothrombin. Fortunately, however, Wohlisch[7] had written an excellent review which summarized the earlier literature on blood clotting. For the first time, I became familiar with the classical theory of Morawitz and of Fuld and Spiro. Gradually, the confusion cleared as I became converted to the creed of the classical theory. The bewilderment encountered in reviewing the literature was no worse than my own groping in the laboratory Dr Stanley-Brown, Mrs Breitung, and I began to study various problems such as the effect of splenectomy on the clotting time, the changes in the blood caused by injection of heparin, and the relation of prothrombin to the end piece of complement. While we made a few interesting observations, little real progress was forthcoming. The clotting time of recalcified plasma continued to interest me and after I became familiar with the classical theory, the well-known equation

Thromboplastin + Ca + prothrombin = thrombin

made its appearance in my notebook. As one trained in fundamental chemistry, I noted the 3 variables in the equation and recognized that only calcium was controlled in the clotting time of recalcified plasma The need to find a suitable thromboplastin reagent was my first concern The only materials that were readily available were the tissues of small laboratory animals. It was indeed lucky that the first material I tested was rabbit lung which I found had a relatively high thromboplastin activity.

On January 6, 1934, I observed the first abnormally prolonged clotting time with the test It was on a plasma from a jaundiced patient. Two days later I obtained similar results on a second jaundiced plasma. I did not attach too much significance to these findings, since the results were often erratic: nevertheless, I made this notation on January 30, "I am still interested in a direct prothrombin test by adding lung extract directly to plasma." I continued to have difficulty with lung extract as a thromboplastin reagent and I soon found that rabbit brain gave more constant results. To preserve the material I spread it on a glass plate as a thin paste and air dried it; from this the extract was made.

Gradually, I began to recognize the possible importance of the new test and was anxious to apply it clinically. Dr. Bancroft arranged with various hospitals (Mount Sinai, Lennox Hill, Doctors', Presbyterian, and St. Luke's) to allow me to collect blood from their jaundiced patients. In a short time through excellent cooperation we had a series of cases of obstructive jaundice with definitely prolonged clotting times as measured by the new test. We also had the opportunity to study a number of hemophilic patients, and found that their clotting times were consistently normal. These results with a description of this new test were presented in a paper which we submitted to the American Journal of Physiology in the early summer of 1934. After 2 months it was returned with the comment that 3 editors had agreed that the paper was "not acceptable." Ten years later the American Medical Association awarded a gold medal for our exhibit that was based essentially on the material contained in that article.

Because of the uncertainty of further financial support of the grant, I left New York in November 1934 to enter private practice in Milwaukee Thus, the first phase of the development of the 1-stage prothrombin time

was completed. Sufficient evidence had been gathered to show that the test had possible clinical usefulness. A report[8] of the work was published in April 1935 With this, the curtain dropped and my pleasant and stimulating association with Dr. Bancroft and Dr. Stanley-Brown was terminated.

Act I, Scene 2

The second scene was laid at the State University of Iowa. Dr. Harry Smith who, as a medical student, had worked on plasma proteins under the guidance of Dr. George Whipple became interested in fibrinogen. Later, in collaboration with Dr. T. B. Jones, he studied the fibrinogen level of the blood after hepatectomy. This brought him into the orbit of blood clotting. With his associates Brinkhous and Warner, he organized an extensive research program. One of their first important contributions did much to reestablish faith in the classical theory. Wooldridge,[9] later Nolf,[10] and in this country, Mills,[11] had built an elaborate theory in which tissue coagulins independent of thrombin occupied a central position; in fact, thrombin was regarded as a by-product. When Smith and his co-workers[12] showed that an extract of lung carefully freed of blood did not clot fibrinogen, it became clear that tissue coagulins were merely mixtures of prothrombin and thromboplastin, and that thrombin was the sole agent that converted fibrinogen to fibrin

As a sequela to these studies, Smith and his associates[13] developed a quantitative method for prothrombin Their method likewise was based on the classical theory and they, too, added an excess of a tissue extract to convert prothrombin to thrombin Instead of measuring the evolution of thrombin directly, however, they devised a procedure in which the conditions were such that the total amount of thrombin formed was measured This required, first, defibrination and then dilution of the plasma before adding a mixture of lung extract, calcium, acacia, and buffer to generate thrombin. The latter was determined by the speed with which it clotted a standardized solution of fibrinogen To distinguish the 2 methods, mine was called the 1-stage test while that of Smith, the 2-stage, though actually this was a multistage procedure. The 2 methods, developed independently and almost simultaneously, agreed remarkably well as measures of the hypoprothrombin in vitamin K deficiency, as well as in other states Differences, however, were encountered and only after many years did it become apparent that the methods did not always measure the same clotting factors and that neither test was an infallible determinant of prothrombin. In the hands of Smith, Brinkhous, and Warner, the 2-stage method supplied much valuable information on prothrombin. One of the casualties of World War II was the disruption of the Iowa team. Eventually, Dr. Seegers, who had joined the group in 1937, became the chief custodian of the 2-stage method and its citadel was moved across Lake Michigan to Wayne University, at Detroit.

Act II

The drama of the 1-stage method shifted to a new scene—a laboratory in the Department of Biochemistry at Marquette University School of Medicine put at my disposal by Dr. Joseph Bock. Since my medical practice did not encroach much upon my time, I was again able to resume active work on tissue thromboplastin. I had noted in my earlier study that the common lipid solvents with the exception of acetone impaired the thromboplastin activity of the material. This clue enabled me to develop a method[14] for the dehydration of rabbit brain with acetone. In this manner a product was obtained which not only had a high and remarkably constant activity but also possessed stability. With this agent a prothrombin time of 12 seconds on human plasma has been consistently obtained in our laboratory for the past 20 years and, interestingly, preparations made in 1938 and kept at room temperature in evacuated ampules have retained full activity until now. Having achieved the goal of producing a thromboplastin reagent of constant activity, it was possible to construct a curve relating the prothrombin time to prothrombin activity.[15] The well-

known hyperbolic curve could be expressed by the equation

$$\text{Prothrombin activity} = \frac{K}{p\,t - a}$$

With a prothrombin time (*p t*) of 12 seconds, the values of the constants K and a were established as 330 and 8 7, respectively.

While perfecting technically the 1-stage procedure, I was on the alert to find opportunities for applying the test In this quest, June 9, 1936, was indeed a memorable day I took a drive to Madison and visited my former teacher, Professor E B. Hart, who told me of the interesting work Almquist[16] was doing on a new dietary deficiency disease that gave rise to a bleeding tendency and also of the studies in their own department on a bleeding condition caused by spoiled sweet clover hay. Later that day I learned more about this interesting disease from Dr Link, who was working on the isolation of the toxic principle of this hay, and from Dr. W K. Smith, who was interested in the genetic aspects of sweet clover. In the course of our conversation, I acquainted them with my prothrombin method and voiced the wish to apply the test to this bleeding condition Dr Smith offered to send me a bag of this toxic hay. None of us, I am sure, realized the far-reaching consequences that resulted from this mutual exchange of ideas and materials

Immediately on my return to Milwaukee, I prepared the special diet of Almquist and began feeding it to newly hatched chicks Soon after the bag of spoiled sweet clover hay arrived, I started feeding it to several rabbits Much to my delight a striking increase in the prothrombin time occurred in the chicks and also in the rabbits In both a bleeding tendency manifested itself when the prothrombin time became moderately prolonged This was probably the first time that the results of a clotting test were quantitatively correlated with a bleeding tendency. I found that the addition of as little as 1 per cent of alfalfa meal to the Almquist diet greatly shortened the chicks' prothrombin time and cured their bleeding I further noted that when the toxic hay was mixed with alfalfa meal, it no longer caused hypoprothrombinemia These findings helped to explain the hypoprothrombinemia which we had observed in jaundiced patients, namely, that a vitamin K deficiency, probably caused by faulty absorption due to the absence of bile in the intestines, resulted in an inadequate production of prothrombin.

The results of these studies as well as the important contributions of Smith and his coworkers established the validity of the classical theory more firmly than ever. For a brief period there was general agreement as to the basic clotting reaction, but this halcyon interlude was of short duration. A new era of turbulence began which was at least in part set off by a simple experiment done with the 1-stage method, the results of which I published in 1943[17] On adding stored human plasma that had a prothrombin time of 40 seconds to an equal volume of plasma from a dog given Dicumarol, which likewise had a prothrombin time over 40 seconds, I obtained a mixture that had a prothrombin time of 10 seconds. This mutual corrective action clearly indicated that the clotting factor lost during storage was different from the one decreased by Dicumarol and that what had been called prothrombin was actually an activity depending on at least two factors[18] This discovery of a new factor made the classical theory no longer tenable. Its foundation had begun to be undermined, and the method that was largely responsible was the simple 1-stage prothrombin time that owed its very existence to this theory

Epilogue

With the passing of the classical theory, a notable period in the study of the clotting of blood came to an end and an exciting new era had its beginning. Interestingly, many of the new methods which are at present extensively employed, such as the prothrombin consumption time, the partial thromboplastin test, and the thromboplastin generation test, are direct outgrowths of the simple 1-stage prothrombin time and are based on the same fundamental principle—measurement of the speed of thrombin production. The principle of the

2-stage method likewise continues to serve as an important tool for research in coagulation. Gradually, it is being recognized that the 1- and 2-stage procedures often complement each other. Both continue to supply fuel for the fire that should light the way for a better understanding of the complex coagulation mechanism.

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The Discovery of Dicumarol and Its Sequels

By Karl Paul Link, Ph.D.

THE STORY of Dicumarol* has been told several times by me in the past 17 years, and often by others. Like any good story it need not be told in exactly the same manner each time In Wisconsin it has become a kind of legend. I shall consider only the high water marks of certain chapters

Fortunately the basic scientific facts on the discovery and development have already been thoroughly recorded[1-4] so that little new information on Dicumarol and its sequels needs to be revealed here. However, when I do introduce new material it will be restricted to that which is documented or sustainable via memoranda or letters

The story begins some 36 years ago on the prairies of North Dakota and in Alberta, Canada. In the 1920's a new malady of cattle involving fatal bleeding showed up almost simultaneously in these areas The veterinarians, Schofield and Roderick, were forced to conclude that the cause of the disease was neither a pathogenic organism nor a nutritional deficiency. The origin of the new malady was traced to stacks of sweet clover hay mysteriously gone bad. Hence the disease became known in veterinary practice as "sweet clover disease" and it was found that it was caused *only* by improperly cured hay made from the common varieties of sweet clover When first observed this disease was in a sense without parallel in animal pathology or human medicine When cattle or sheep ate the spoiled hay the disease slowly became manifest by a progressive diminution in the clotting power of the blood (about 15 days) and resultant internal hemorrhage which usually became fatal in about 30 to 50 days

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It was recognized by Schofield and Roderick that the disease was reversible It could be controlled in cattle by the withdrawal of the spoiled hay from the diet and by transfusion of blood freshly drawn from normal cattle, provided the hemorrhagic extravasation had not proceeded too far. Indeed, they showed that even in desperate cases, recovery could be hopefully anticipated after transfusion and change in diet (good hay)

In a comprehensive and thorough study of the pathology and physiology of the disease Roderick in 1931 emphasized that the delayed or abolished coagulability of the blood was due to a "prothrombin" deficit Indeed he showed that the severity of the hemorrhagic condition paralleled the reduction in "prothrombin content or activity." He did this by using the technic developed by that great American pioneer of blood coagulation, the late Professor W. H. Howell. Solutions of what was then called "prothrombin" prepared by precipitation of normal bovine plasma with Howell's acetone method when added to the "sweet clover blood" promoted coagulation In contrast, preparations of "prothrombin" made in a parallel manner from "sweet clover blood" did not produce coagulation. The other constituents for the maintenance of normal coagulability known at that time (fibrinogen, calcium, platelets, and inhibitory substance) appeared to be unaffected.

I first learned about the hemorrhagic sweet clover disease of cattle in December 1932 through the late Ross A Gortner, who then

*Dicumarol is the trademark for 3,3'-methylenebis-(4-hydroxycoumarin). The anticoagulant was made available in 1940 and 1941 for clinical use by the cooperative efforts of the Wisconsin Alumni Research Foundation, Madison, Wis, the Abbott Laboratories, North Chicago, Ill, Eli Lilly and Company, Indianapolis, Ind., and E. R. Squibb and Company, New Brunswick, N. J. The official U.S.P. name is bishydroxycoumarin

headed the Biochemistry Department of the University of Minnesota. He had offered me a post and I had come to St. Paul to consider it. Since the "sweet clover disease" was also a problem in Minnesota it was one of the projects open for study if I chose to accept. It was Gortner who supplied me with the original publications of Roderick. Some attempts had been made in Gortner's department to extract the hemorrhagic agent but they, like those of Roderick and others, had failed.

Curiously, the "official" start of *our* work in January 1933 in cooperation with Professor R. A. Brink and W. K Smith of our Genetics department was on a different aspect of the sweet clover problem. They sought to develop a strain of sweet clover suitable for Wisconsin climatic conditions low in, or free from, coumarin. Though coumarin smells sweet (the characteristic smell of new mown hay is due to its presence) it tastes bitter, and it was known that the bitter taste of green sweet clover plants, Melilotus alba and M. officianalis, paralleled the total coumarin content. In actual practice it was observable that cattle (or rabbits) would eat the less bitter plants first.

Tragedy out on the Farm

Quite apart from the "official" start concerned primarily with the palatability question my laboratory had a direct catalytic hit from agricultural practice.

Indeed on a Saturday afternoon in February 1933 following the first conferences with Brink, while a blizzard was howling and the mercury was hovering near zero, a farmer from the vicinity of Deer Park, Wisconsin, some 190 miles from Madison appeared with what the late Professor A J Carlson might have called "the evidence" Curiously the farmer's name was Ed Carlson The hemorrhagic sweet clover disease of cattle was rampant on his farm He had fed sweet clover hay for years previously without encountering any difficulties and he doubted the veterinarian's diagnosis Accordingly he was advised to go to the Agricultural Experiment Station authorities to get the facts The office of the State Veterinarian had closed and pure chance had brought him to the Biochemistry Building

Farmer Carlson's multiple evidence was a dead heifer, a milk can containing blood completely destitute of clotting capacity, and about 100 pounds of spoiled sweet clover—the only hay he had to feed his cattle.

His account of the over-all course of the disease coincided perfectly with the classical "sweet clover poisoning" picture. Late in December he had lost 2 young heifers In January 1 of his favorite old cows had developed a massive hematoma on a thigh and following a skin puncture fatal bleeding set in rapidly. Finally 2 young cows had died on Friday and the bull was oozing blood from the nose. So he took off for Madison in a blizzard.

I immediately had to tell farmer Carlson that we could do no more at this time than to recommend the teachings of Roderick and Schofield. He had to stop feeding *that* hay, and possibly transfuse those desperately sick cattle, if he wanted to save them Eventually it might become possible to make some usable recommendations to avoid such disasters, but not now.

I can still see him take off for home about 4:00 p.m. Those 190 miles of drifted roads between our laboratory and his barn must have appeared to him like a treacherous and somber ocean.

I cannot take the time to tell all the details of this slice of the Dicumarol story, but I can assure you its impact on me was immense. I will relate a part of it exactly as I did in my first lecture on Dicumarol given at the Mayo Clinic on March 12, 1942.

When farmer Carlson came to see us, my senior student and old man Friday was Eugen Wilhelm Schoeffel, a volatile Schwabian who came to the U.S in 1926 with a diploma in Agricultural Chemistry. After serving a 2-year apprenticeship in the Chicago Stock Yards he began to study with me in 1929. Schoeffel is interesting, energetic, and loyal He was then and still is, somewhat of a mystic and inclined in ordinary conversation to quote freely from Goethe's Faust, Shakespeare, and the Bible, as well as other primary sources. In 1933 his spoken English was not only strongly guttural, but also very earthy, punctuated frequently with Schwabian German.

After farmer Carlson left, Schoeffel stormed back and forth in the laboratory shouting, "Vat da Hell, a farmer shtruggles nearly 200 miles in dis Sau-wetter, driven by a shpectre and den has to go home vit promises dat might come true in five, ten, fifteen years, maybe never. Who knows? 'Get some good hay—transfuse.' Ach!! Gott, how can you do dat ven you haf no money?" he snarled.

He dipped his hands into the milk can repeatedly and while rubbing them muttered, "Dere's no clot in dat blook! BLUT, BLUT VERFLUCHTES BLUT. 'Die Menschen dauern mich in ihren Jammertagen.'" (Faust Prolog, line 297) and then, "Vat vill he find ven he gets home? Sicker cows And ven he and his good voman go to church tomorrow and pray and pray and pray, vat vill dey haf on Monday? MORE DEAD COWS!! He has no udder hay to feed—he can't buy any. And if he loses de bull he loses his seed. Mein Gott!! Mein Gott!! Vy didn't ve anti-shi-pate dis? Ya, ve should haf anti-shi-pated dis"

We took the blood and hay and played about with them until about 7 00 p.m. when I headed for home As I left the laboratory, Schoeffel grabbed me by the shoulders, looked me squarely in the face and said, "Before you go let me tell you something. Der is a deshtiny dat shapes our ends, it shapes our ends I tell you! I vill clean up and gif you a document on Monday morning"

Development of the Bioassay

Two fundamental issues confronted us First there were no *chemical* criteria available to establish the presence of the hemorrhagic agent. Therefore, a *bioassay* involving a small experimental animal (rabbits)) offered the only practical means of appraising the anticoagulant activity of test hays and extracts prepared therefrom.

It was clear from the pioneer papers of Roderick that the sweet clover disease *was completely reversible*. The eating of spoiled hay, even over long periods, caused no permanent functional change, no demonstrable morphologic change, and no detectable pathologic change of the liver, the assumed primary site of prothrombin synthesis. Nevertheless, the immediate prospects of developing a reliable and simple bioassay were not bright; indeed they were dark, "dark like the inside of a cow." We had not had previous experience with that complex problem—blood coagulation.

Schoeffel and Roberts first showed that the Howell method for estimating prothrombin activity did not have the precision required. Smith and Roberts showed that the whole blood coagulation time was too variable, and that the Quick 1-stage method using whole plasma left much to be desired Smith also showed that there was a wide variation in the response of individual rabbits to the standard dose of 50 Gm of the spoiled hay. So Campbell and Smith bred and reared a susceptible rabbit colony specifically for the assay.

At that time, 1935-1938, a bloody and amusing polemic raged among the coagulation specialists on how to estimate "prothrombin concentration or activity"—whether it should be done by the 1-stage method of Quick* or the 2-stage method of H P. Smith and coworkers[1-3, 8] We tried to keep out of that brawl In 1938 Campbell finally got over the chief obstacles He adapted the Quick 1-stage method to our conditions, primarily by relying on the clottability of diluted plasma within the concentration range 12.5 to 8.34 per cent. He eliminated some of the inherent daily variations by fasting the assay rabbit 24 to 36 hours before feeding any preparation under test, by making the plasma clottability tests promptly after drawing the blood, and by comparing the test plasma against the normal plasma of each rabbit

Through the use of individually standardized rabbits (the standard response being that

*The intricacies of the blood coagulation phenomenon are outside the scope of this discourse Suffice it to state that it is now accepted by most "coagulationists" that a prolonged Quick 1-stage "prothrombin time" (when the fibrinogen is normal) induced by Dicumarol and the like is a primary deficiency in factor VII and prothrombin See British Medical Bulletin, vol II, no 1, Blood Coagulation and Thrombosis, Medical Department, The British Council, London, November (1955), and the lectures by Owren P. A. on Coagulation of Blood, etc, Northwest Medicine, January, pp 31-39, February, pp. 159 166, and March, pp 298-307, 1957.

induced by the anticoagulant in 50 Gm. of spoiled hay) and by having the assay on a strictly differential basis the ever present problem of biologic variation was greatly reduced.

Some side observations were made by Campbell on the plasma of rabbits fed the spoiled hay or fractions thereof that were later reported by others A plasma factor beyond that needed by the classical blood coagulation expression of Morawitz-Field and Spero was nted at in one of Campbell's reports. But iese hares were not hunted. Our goal was o make real a substance that abolished the lottability of cattle blood in agricultural practice. To use the vernacular, the bioassay using the 1-stage plasma clottability was altered so that "it worked," and few of the valuable assay rabbits were lost in the process. One of them known as Bess Campbell was used for about 200 individual assays, over a period of 5 years.

Isolation, Crystallization, Identification, and Synthesis of Dicumarol

Between that fateful Saturday in February 1933 and June 1939 a long and arduous trail was followed by Smith, Roberts, and especially Campbell, to lay the anticoagulant out on the bench. I would like to detail some of the chemical extraction, separation, and isolation problems that the spoiled sweet clover hay presented This hay was indeed a kind of biochemical grab-bag and yielded many inacve products, some new, most of them old ut suffice it to state that many a seething nd simmering hope did not become reality. At times the hemorrhagic agent appeared to hover before us like thistle down only to elude us like the will-o-the-wisp At one time it was thought to be a porphyrin-like substance, a pheophytin resulting from the degradation of the chlorophyll in the spoiling process

Finally in the dimness of dawn on June 28, 1939, after working all night, Campbell saw on a microscope slide what turned out to be crystalline Dicumarol. Two hours later he had collected about 6 0 mg of it

When I reached the laboratory that morning Campbell was asleep on the laboratory couch; the door to the room was guarded by one Chet Boyles, a soldier of fortune on the W.P.A. relief roles, who assisted Campbell with the bioassays. Boyles was an excellent handler of animals for he had served 2 years as helper to a veterinarian before he came to us.

As I walked into the room, Boyles was taking a nip from the contents of a bottle whose bottom layer consisted of carpet tacks, the upper layer of 95 per cent ethanol Without the flicker of an eyelash Boyles said to me, "I'm celebrating, Doc. Campy has hit the jack-pot." (As though I didn't know that he had been hitting that bottle for months)

But Boyles' surmise was correct this time. Campbell did have Dicumarol and the first bioassay to establish its anticoagulant potency was already in process!

Campbell avoided me for 2 days—until the results of the assay were available—and then he came in to report.

There is a bed-rock of matter-of-fact common sense in Campbell's makeup. He was not inclined to show his emotions, but it was apparent that he was secretly as happy as a boy who had just caught his first big fish He passed the vial to me and said, "This is H A.!" (H. A. was the laboratory code for hemorrhagic agent) I did not disclose that Boyles had given me the tip-off. I told Campbell that I knew a couple of lines of German poetry that fitted the occasion, and I recited to him,

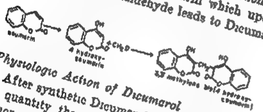
> "So halt'ich's endlich denn in meinem Händen
> Und nenn' es in gewissen Sinne mein"

We sent a short wire to Schoeffel, who was then in the control laboratory of the American Medical Association in Chicago He responded at once with a 200-word reply wherein he expressed his *complete* confidence in Nature, Fate, and us.

Mass isolation was started at once, and a stock of about 1,800 mg. of the crystalline anticoagulant was accumulated (Stahmann).

The problem of determining its structure fell to the sensitive, brilliant, and deft C. F. Huebner, who with some assistance from his lively imagination made the correct structural diagnosis as 3, 3'-methylenebis (4-hydroxycoumarin). He set the sights for the synthesis, which was achieved on April Fool's day, 1940. The synthetic and the natural product were shown to be chemically identical. Subsequently, Overman and Sullivan, through carefully conducted tests on the rabbit, rat, guinea pig, mouse, and dog, hall-marked the natural and synthetic products as biological equals

The determination of the structure of the anticoagulant as a 3-substituted derivative of 4-hydroxycoumarin makes it appear that both of the undesirable aspects of the common sweet clovers—their unpalatability (bitterness) in the green state and the tendency of the hays to cause hemorrhage when improperly cured—have a common basis in the coumarin molecule The biological synthesis during spoilage can be rationalized as an oxidation of coumarin to 4-hydroxycoumarin which upon coupling with formaldehyde leads to Dicumarol



Physiologic Action of Dicumarol

After synthetic Dicumarol became available in quantity the essentials of its physiologic action were quickly established It was shown that there is a lag in response, a variation in the intensity and duration of the hypoprothrombinemia (plasma prothrombin clotting time), depending on the size of the dose In each species tested a certain single dose level gives the most efficient response Below this level the efficiency of action is decreased by a threshold effect and at high levels by incomplete absorption of the drug

Due to the latent or lag period of 12 to 24 hours before the drug's action becomes apparent, there is a cumulative effect following repeated administration Thus it was anticipated that in clinical practice this action will vary with the individual and because of this variation optimal therapeutic effects without hemorrhage would be obtained only when the dosage is individualized

A brief summary[1-8] of the details follows:

1 There is a wide species difference in the response induced in the rabbit, rat, guinea pig, mouse, dog, cat, and chicken, and this varies with the age and sensitivity of each individual. Broadly speaking, the rat and mouse are the most sensitive, the cat and dog intermediate, and the rabbit, the cow, and the chicken the least sensitive

2. The vitamin K and C levels in the diet affect not only the intensity but also the duration of the anticoagulant action I propose to elaborate on this later

3. The nutritional status of the animal affects the anticoagulant response—fasting generally enhances it in all species.

4 Any pre-existing hypoprothrombinemia like that inducible by the salicylates (aspirin), the sulfa drugs, or mild chloroform anesthesia *augmented* the response

5 The hepatic and renal function influences both the intensity and duration of the response.

6 The presence of drugs that affect the total functioning capacity of the liver, like the methylxanthines (theophyllin) and the digitalis drugs, have a mild but definitely detectable counter action

7. Pregnant or lactating females show a slight resistance to the drug's anticoagulant action

These observations did not exhaust the conditions that can influence Dicumarol's action but they cover the essential points. Finally, it should be added that in Dr. Best's department at Toronto, Dale and Jaques first[9] and later Meyer and co-workers[10] at Wisconsin General Hospital, and others[11-13] were able to show that a primary relationship exists between thrombus formation and the clotting mechanism of the blood These studies established for the first time that an effective reduction of extravascular and intravascular thrombus formation parallels the diminished hypocoagulability induced by Dicumarol. It

was also shown by Spooner and Meyer[14] that, when Dicumarol is given to dogs in safely usable therapeutic doses, it definitely decreases platelet adhesiveness; at the same time Quick showed that it also reduced platelet agglutinability.[13] Thus the clinical use of the anticoagulant as a prophylactic agent for (against) thrombosis rested on a sound experimental basis.

Breaking the Bonds of the Usual Pattern of Thought

When we turned Dicumarol over to the clinicians in the years 1940 to 1942, one significant point, clearly established by our work, was at first missed, in fact denied.* I have reference to the capital fact that vitamin K (all forms—some better than others) can counteract the action of Dicumarol.* I emphasized this in letters, personal conversations, and in my first lecture on Dicumarol at the Mayo Clinic and at Wisconsin General Hospital. In spite of these efforts the first clinical reports carried the statement that "vitamin K has no effect as an antidote to the administration of Dicumarol." The editorial and annotation writers for the medical journals, those who only "think" but "don't try," innocently reiterated this statement.[1,2] While in error, the clinicians were in good company, for an authority on blood coagulation[16] had written in 1937, and again in his book published in 1942, that "vitamin K will not restore the prothrombin concentration" depleted by Dicumarol.[17]

Originally these denials made me very unhappy. The misfortune of being accused of error was not the primary basis for the unhappiness, for we were certain that the antidotal capacity of vitamin K would in time be sustained in the clinic. What did disturb me was the needless induction of the hemorrhagic "sweet clover disease" in man and the stigma temporarily attached to Dicumarol, that it was a dangerous drug.[18] *And this did happen.*

A feature of science that has always appealed to me is that sooner or later, and usually sooner, "the truth will conquer."

Dr. Shepard Shapiro in New York City was the first clinician (February 1942) to sustain our claims that vitamin K can counteract the anticoagulant action of Dicumarol in man when liver function is adequate.[5,20] Subsequently he was independently supported by Townsend and Mills in Canada,[21] Lehman in Sweden,[22] and finally by Cromer and Barker[23] at the Mayo Clinic, as well as others. Today it is accepted that the water-soluble forms of vitamin K or vitamin K_1 given orally can successfully antidote overdosing with Dicumarol, provided they be employed when reversal is still possible.

Let us briefly examine why the error arose. The clinicians did not use a 1-stage prothrombin assay as sensitive as the one Campbell developed for our experimental animals. They were originally conditioned to the low levels of vitamin K effective in obstructive jaundice, biliary fistula, cholemic bleeding, etc. It was also thought that the menadione form of vitamin K might be toxic. Over 10 years were required to wipe out this error from clinical practice.

To summarize, surmise, faulty thinking, and not enough trying kept vitamin K from being the corner building stone in Dicumarol therapy that it deserved to be from the outset.

In 1950 Marple and Wright (pages 149 and 181)* wrote, "When bleeding occurred the fault rested with the physician who administered the drug."

Enthusiasm—Muddle—Consolidation

Within 2 years after Dicumarol was synthesized, over 100 related 3-substituted 4-hydroxycoumarins were prepared in my la[b]

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*For an account of how, in January 1939, a bull desperately sick from eating spoiled sweet clover hay (he was "down," blood was oozing from the nose, and a massive hematoma adorned the right thigh) was rescued from the clutches of death via a vitamin K_1 concentrate prepared from alfalfa hay, see reference 2. Originally not even this "bull story" could break the bonds of the usual pattern of thought. The equation is

$$\text{Normal bull} \underset{\text{Vitamin } K_1 \text{ concentrate}}{\overset{\text{Spoiled sweet clover hay}}{\rightleftharpoons}} \text{Bleeding bull}$$

a completely reversible reaction

oratory. Synthesis ran substantially ahead of biochemical appraisal. Accordingly, when I gave the Harvey Society lecture on "The Anticoagulant from Spoiled Sweet Clover Hay" in January 1944[1] it was indicated that "it would not be valid to conclude from the relative appraisals on activity made with the rabbit—that Dicumarol is the most desirable compound for clinical use" It was indicated that "In the course of the routine appraisal of the many compounds tested it was learned that some of them exhibited a slower but more sustained hypoprothrombinemic action, while the action of others is of shorter duration It will take some time before final judgment can be passed on this subject. From the experience gained with other pharmacological agents it is abundantly clear that the final test is the action in man under a variety of conditions The unpredictable can be surprising, so, as we see it, we might now be at the beginning of things and not at the end in this field of study."

Being an agriculturist I have little confidence in predictions, including my own The situation can now be appraised in the light of wisdom after the event Bear in mind that the statement quoted was made less than 4 years after Dicumarol became known to us and before extensive clinical information on the response in man was available. About 50 reports on the clinical use of Dicumarol had appeared between 1941 to 1944[5, 6*]

The appearance of any new drug creates an interesting cycle of events, and Dicumarol went through that cycle quite rapidly. The first preliminary reports indicated that an atmosphere of optimism prevailed They evoked prompt favorable editorial comment in the Lancet (September 13, 1941) under the title, "*Heparin and a Rival*" Then came the second period—a period of muddle Enthusiasts and skeptics for anticoagulant therapy with Dicumarol were created, and it can be stated that some of the skeptics condemned the drug in no uncertain terms, though they were largely armed with surmise, faulty, or no prothrombin clotting time determinations and they used the antidote vitamin K inadequately Then came the third period of consolidation, from which it can now be concluded that a better anticoagulant of the Dicumarol type was desired

Since Dr. Wright asked for aspects of human interest, let me add another slice from the Dicumarol story. Early in September 1945 I was fed up with laboratory work, etc, and I went off on a canoe trip with my family. On this trip we were caught in a cold rain storm. I got soaked and overexhausted Two weeks later I came down with what I had had once before—after a similar heavy physical bout, as a student in Switzerland—wet pleurisy. At first my doctor thought I had pneumonia; then I told him about my previous bouts of tuberculosis; so the diagnosis was changed to reactivated pulmonary tuberculosis I spent 6 months at Wisconsin General Hospital and then was transferred to Lakeview Sanatorium headed by the double cross of Lorraine Here I was supposed to vegetate like a topped carrot I did rest there, physically for 6 months, took nothing stronger than cod liver oil and 3 bottles of beer a day, but kept the aged tuberculosis out of my mind by studying laboratory records and reading the history of rodent control from ancient to modern times.[24, 25]

A "Janus" in the Coumarin Family

Now brace yourselves, for I propose to shift from a "cow poison" that had become a drug of substantial clinical usefulness, to a "rat poison" converted to a drug, which has I believe most of the desirable features that can be expected from an anticoagulant to be given primarily via the oral route.

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*The first clinical report to appear was by Butt, H R, Allen, E V., and Bollman, J. L.: *Preparation from spoiled sweet clover (3,3'-methylene bis-4 hydroxycoumarin) which prolongs the coagulation and prothrombin time of blood: Preliminary report of experimental and clinical studies*, Proc. Staff Meet Mayo Clinic 16: 388-395 (June 18), 1941. See also Allen, E V, Barker, N W., and Waugh, J M, J A.M A. 120 1009, 1942; Wright, I. S, and Prandoni, A, J A M.A 120: 1015, 1942, Bingham, J B, Meyer, O O, and Pohle, F J, Am J. M Sc 202. 563, 1941

The many coumarins synthesized between 1940 and 1944 were listed by numbers in logical groups based on their chemical structure.[1] While I was in the sanatorium in 1945-1946 the laboratory work was practically at a standstill. There were few students available, since most of them were still in the armed forces. So I had ample time to reexamine all the chemical and bioassay data available. Upon the return of L. D. Scheel from service in the spring of 1946, he was assigned to the task of reappraising the anticoagulant activity of the compounds numbered from 40 to 65. They were made by Ikawa in 1942-43. Instead of using only rabbits for the bioassays Scheel also used rats, mice, and dogs. In 1946-1948 he defined coumarin numbers 42 and 63 as being much more potent than Dicumarol in the rat and dog, as capable of producing a more uniform anticoagulant response, and as having the quality of maintaining a more severe state of hypothrombinemia without inducing visible bleeding. Certain chemical properties were also considered the degree of purity readily attainable (absence of taste and odor), the cost of making them, and the property of being convertible to stable water-soluble salts.

Back in 1940 to 1942, Overman, Field, and my colleague, C. A. Bauman, had studied extensively the action of Dicumarol in the laboratory rat, and the effect of diet on the response, specifically the influence of vitamin K and foods rich in it. Later in 1942 I personally, with the help of good old Schoeffel, set up field trials to ascertain the suitability of Dicumarol for rodenticidal purpose. It was concluded that the activity of Dicumarol in the rat was not high enough to make it practical for rodent control This was found to be largely due to the vitamin K content of mature grains and the availability of green foods with a high vitamin K content It was shown that rats could tolerate a daily intake of 2 0 mg of Dicumarol for 60 or more days due to the vitamin K content of the natural foods available On a semisynthetic diet essentially free from vitamin K the survival time was about 15 to 23 days When 5 mg. of vitamin K per day were added to the artificial diet, the rats also tolerated 2 0 mg. of Dicumarol daily for over 60 days

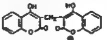
Early in 1948 I told Scheel and Dorothy Wu that I wanted to propose no. 42 for rodenticidal use.[24, 25] This proposal shook the laboratory. I can sum up by stating the consensus of opinion "the boss has really gone off the deep end this time." Scheel favored no. 63 for clinical purposes They are chemically closely related, no. 63 being a direct derivative of no. 42. To make a long story very short, early in 1948 no 42 was promoted for rodent control under the auspices of the Wisconsin Alumni Research Foundation through the able, enthusiastic, and public-spirited Ward Ross, General Manager of this organization. Within a short time this effort revolutionized the art of rodent control (multiple doses as opposed to the single dose of the highly toxic poisons), and warfarin rapidly became and still is the leader in the rodenticide field.* The name Warfarin was coined by me by combining the first letters of the Wisconsin Alumni Research Foundation with the "arin" from coumarin—and it is now a household word throughout the world.**

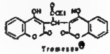
Between 1948 and 1952 Dicumarol was, so to speak, being squeezed by chemical kin stemming primarily from European studies[26] "Imitation is the sincerest form of flattery." Curiously, one of them, a derivative of Dicumarol, trade-named Tromexan, was not seriously considered by us as early as 1940 Though it acted somewhat faster than Dicumarol, it required substantially larger doses

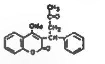
*Just as cattle eat hemorrhagic sweet clover hay until they die without visible sensory responses, the rat eats warfarinized cereal grain bait until fatal hemorrhage sets in. Neither bait refusal nor bait shyness develops Indeed, the rodent's departure is biblical "death without sting ,, In Maxwell Anderson's drama, Elizabeth the Queen says, "To the end of time it will be so . . . the rats inherit the earth ,, Since warfarin has become available, this need not be so Furthermore, via the water-soluble warfarin sodium the rat can drink unto death.

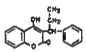
**Warfarin is the safest rodenticide known Up to now, in the United States there is no recorded case of a warfarin induced fatality in man, although over 140,000,000 pounds of warfarin containing bait (0 025 per cent) have been distributed since 1950

to get the equivalent anticoagulant action.* The second, Marcumar, a close kin to warfarin, was also passed by us, since its water-soluble sodium salt is less stable than warfarin sodium. Milligram for milligram, Marcumar is more active than warfarin and its action is also more prolonged But as a result of the claims made about Tromexan and Marcumar I took another good look at the mass data in the light of what clinicians were seeking, namely an anticoagulant that could be used via any route with the retention of the virtues of Dicumarol but without its limitations.


Dicumarol®


Tromexan®


Warfarin Sodium
(Coumadin Sodium®)


Marcumar®

Based in part on the supposition that the response of the rat to warfarin (no. 42) was a very reliable index of how man would respond, late in 1950 I told Dr. S Shapiro and Dr. O. O. Meyer that the water-soluble sodium salt of warfarin should be tried on man In 1941 the clinicians had literally snatched the "cow poison" from us, but the transition to a substance originally promoted to exterminate rats and mice was a bit more than they could accept with real enthusiasm. Then, on April 5, 1951, we were informed by Captain J. Love (MC) in the U.S N. at Philadelphia that an army inductee was admitted to the Naval Hospital who had taken over a period of 5 days a concentrate of warfarin designed for rodent control[27] The package contained 567 mg. of warfarin in corn starch. The inductee had followed the *multiple dose directions* on the package It became clear to him that warfarin was not an efficient agent "to shuffle off" this "mortal coil " It allowed too much time for thinking—so he went to the hospital with a fully developed case of hemorrhagic "sweet clover disease." He was treated per the directions—blood transfusion and large doses of Vitamin K—and made an uneventful recovery.[28]

This incident acted as a catalyst. Shapiro[29] and Meyer[31] both concluded from their carefully done work with warfarin sodium that it did possess certain properties not inherent in Dicumarol or the other anticoagulants they

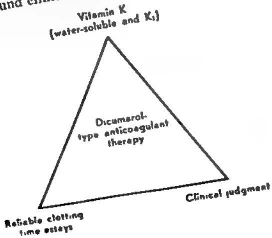
*The clinical promotion of Tromexan (bis-3,3'-4-oxycoumarinyl) ethyl acetate, referred to as B.O E A. in the article by Burt, C. C, Wright, H. P., and Kubik, M, Brit. M J 2 1250, 1949, precipitated interesting editorial comment under the heading, *Dangers of Dicumarol* (pp 1279 1280). In this editorial it was suggested that since Tromexan seemed to be superior to Dicumarol "owing to its shorter-lived action . " and in view of recent reports of the drug's (Dicumarol's) efficiency as a rat poison, it may be that Dicumarol will ultimately be more useful for that purpose "

Unfortunately the significance of our paper on the action of Dicumarol in the rat dealing specifically with the effect of diet and vitamin K on the anticoagulant action (J Nutrition 23: 589 602, 1942) was not appreciated by O'Conner, J A, Research 1: 334, 1948, who suggested the use of Dicumarol for rodent control Had O'Conner read our paper carefully, he would not have made this suggestion The critical issue is that Dicumarol's anticoagulant action in the rat subsisting on natural grain foods is too slow to be practical. The level of Dicumarol in the bait has to be set so high that other animals (cat, dog) and children (accidental ingestion) would be vulnerable

It was the inefficiency and slowness of Dicumarol to kill rats under practical field conditions that caused me not to suggest its use as a rodenticide in 1941-1943 (letter, Link, K. P., to the National Defense Research Council, Washington, D C., dated March 10, 1943, and confidential disclosures, 1942-1943, to the late Professor Homer Adkins and Professor H Gilman, official investigators and project leaders of N D R C and O S R D (confirmatory letter of Gilman to Link, June 11, 1952) Instead of Dicumarol the much more potent and efficient (no. 42) warfarin was recommended. Nevertheless, O'Conner's paper served a useful purpose in rodenticide control circles, and he must be accredited with being the first one to stimulate, via the printed page, the backward pest control workers by pointing out the potentials of anticoagulants (Link, K. P., letter December 6, 1948, to U.S D I. Fish and Wildlife Service, Denver, Colo) I had attempted to create an interest in warfarin via letters and memoranda, which at first failed to reach the objective (see reference 24 and particularly reference 25). A complete history of the warfarin development based on 10 years of practical field experience is in the process of being prepared.

had tried. After Collin Schroeder perfected the process of making warfarin sodium, I induced my long-standing friend, Dr. S. M. Gordon, of the Endo Laboratories, Richmond Hill, N.Y., to make it available for clinical use. This he did, under the trade name Coumadin Sodium. Today it would appear, from the 15 to 20 clinical papers on warfarin sodium that have been published (see reference 32), that most of the drawbacks of Dicumarol have been overcome. Warfarin sodium is at least 5 and possibly 10 times more potent than Dicumarol. It is the only synthetic anticoagulant available today for therapeutic anticoagulation that can be given orally, intravenously, intramuscularly, or rectally.[32] The rate of absorption is almost the same, irrespective of how it is administered. No other anticoagulant of the Dicumarol type has all these virtues. Of course, an overdosage can be readily corrected via vitamin K. It acts faster than Dicumarol, and fewer prothrombin times are required in its routine use. To use the words of both Shapiro and Meyer, "It is easier to handle clinically." It is my firm belief that in time it will replace Dicumarol on the basis of its performance over a wide variety of conditions and that other anticoagulants of the Dicumarol type will not be superior.

It always seems appropriate to me to visualize successful anticoagulant therapy with the Dicumarol-type drugs as being shaped like a triangle with accurate "prothrombin assays" at one corner, vitamin K at another, and sound clinical judgment at the third



Each corner is linked to the other by way of the connecting sides. There should be no separation, each is vitally dependent on the other two. Though the clinical judgment be good and the "prothrombin time" accurate, the vitamin K corner might still have to be evoked, since each individual patient is essentially "an unstandardized biologic entity," errors in dosage can be made by the hospital service, the patient might have a silent ulcer, or the functioning of the *liver* or *kidney* might unknowingly be penumbral.

On September 29, 1955, I got a card from a former Wisconsinite working in Fitzsimons Army Hospital in Denver, Colorado, which read, "The President is getting one of your drugs and it's not Dicumarol." A day later press secretary J. C. Hagerty announced,[33] "The heparin which was used initially as the anticoagulant has been replaced by a drug of the Dicumarol type. The present prothrombin level has been well maintained." I knew of Colonel Pollock's paper, "Clinical experience with warfarin (coumadin sodium a new anticoagulant)," read before the first annual meeting of the American College of Angiology Atlantic City, N.J., on June 4, 1955, and I surmised that the most important man in the world today was being anticoagulated via warfarin sodium.[34] This surmise proved to be correct and since then it is an open secret that warfarin sodium was being used. "*The unpredictable can be surprising.*"

In closing I wish to indicate that what my laboratory has achieved in the past 2½ decades represents the combined effort of many students. It is fun to be the reporter or narrator of this highly successful adventure. To use the words of the late Allan Gregg,[35] my students represented much "emergent ability." I think the secret of their success is 3-pronged: they never ceased to wonder, they kept on trying, and they were on a project directed toward doing mankind *some good* instead of trying to *destroy it*.

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The Introduction of Heparin Therapy in Cases of Early Thrombosis

By Gunnar Bauer, M.D.

THE CURRENT METHOD of combatting acute deep venous thrombosis of the leg by means of very early diagnosis, intermittent intravenous injections of heparin, free movements of the affected limb, and early ambulation was, I believe, first used at the Mariestad Hospital, Sweden, where it was introduced on October 1, 1940. I shall give a brief account of the events that led up to this scheme of treatment.

Being originally interested in the clinical and roentgenologic study of blood vessels, I was impressed by an article published in 1938 by Cid dos Santos,[1] describing a technic for introducing x-ray contrast medium into the deep veins of the leg. After having made some modifications to adapt it for clinical purposes, I found that it provided a reliable method for studying roentgenologically the deep veins of the lower leg in particular. When the normal anatomy had been established, the pathologic changes brought about by thrombosis were studied. It soon became evident that the whole of the thrombotic process, from the first beginning to the final stage, could be followed in a way that had not earlier been possible.[2, 3]

The matter of principal interest in the present connection is the fact that it could be shown that the thrombotic process almost invariably (in 96 to 98 per cent of the cases) originates as a small adherent thrombus in one of the deep venous trunks of the lower leg. At that time, it was generally believed that the process first appeared in the pelvic or femoral veins. That it actually started in the calf was suspected by a few, but no eviden... be produced. With the aid of phleb... the question was now settled beyond ...

Comparing the x-ray findings with clinical data, I was able after some time to single out the faint clinical symptoms associated with the early stages of intravenous thrombosis, and presently I found that it was possible to diagnose leg thrombosis at a much earlier stage than had hitherto been possible. There was no need to wait for pain and milk leg to appear. The process could be detected when it was still localized to the lower leg, i.e., in its statu nascendi.

This was a point won, but it did not bring us one step nearer to helping the afflicted patients. In fact, we found ourselves, in 1939 and the beginning of 1940, in a somewhat anomalous position. Although we knew how to diagnose thrombosis in its initial stage, we were unable to prevent the process from following the path we knew only too well it would take. During this period we were faced on 29 occasions with cases in which phlebography disclosed the presence of incipient thrombosis of the lower leg. Since no effective therapy was available, we had the painful experience of remaining inactive and seeing how, in 24 cases, the process spread after 1 or 2 days to the femoral vein, and brought about a phlegmasia alba dolens. In 5 cases there was propagation to the large pelvic veins, and in 10 cases the other leg as well was attacked by thrombosis. Pulmonary embolism developed in 11 cases, 2 of which were fatal. The result would probabl... the same in another 2 cases with ... infarcts if recourse ... of the femoral ... remark-

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From the Department of Surgery, ... tal, Mariestad, Sweden

It was in this position that we decided to make a trial with heparin.

Concerning this drug, a fair number of facts, exhaustively reported by Jorpes,[4] and Crafoord and Jorpes,[5] had accumulated at that time. Reviewing them, we concluded that prophylactic therapy could be ruled out as a routine method, and also that treatment of fully developed thrombosis had not yielded fully satisfactory results, even if Murray[6] had been fairly successful in a series of cases of embolism or thrombophlebitis. However, being in possession of a method for diagnosing thrombosis at an earlier stage than had formerly been possible, it occurred to us that if heparin were to be introduced at such a stage, better results might be expected. Accordingly, after cooperation with Jorpes and Crafoord as to the size of the heparin doses, we started treatment along these lines on October 1, 1940. Heparin was given intravenously 3 or 4 times a day, the doses varying between 100 and 150 mg in each injection. Free movements of the leg were encouraged, and the patients were allowed out of bed as soon as the acute symptoms had subsided.

The results were favorable from the outset, and the entire course of the disease was found to be completely changed. No spreading of the thrombotic process occurred, and fever, swelling, and pain disappeared in a surprisingly short time. The patients were, as a rule, able to leave their beds in less than a week, completely healed. The mortality at once fell to less than one tenth of the earlier figure.

As the aforementioned mode of action was also found to involve little or no risk to the patient in the form of hemorrhagic or other complications, and because it became evident that the necessary heparin doses could easily be fixed without any determinations of the coagulation time, it was decided to continue along the same lines. Thus, the method has been used for more than 17 years, without any modifications.

During this period, the results have been reported at various intervals.[7-13] At the time of the most recent publication,[13] they were as follows. Heparin therapy was given in altogether 627 cases of thromboembolism. Five deaths occurred; the mortality was thus 0.8 per cent. In 622 cases, the course during treatment was mainly uneventful. The mean duration of recumbency was 4.4 days. Complications were infrequent. A recurrence took place in 17 cases (2.7 per cent) and a slight hemorrhagic tendency was noted in 13 (2 per cent). Pulmonary embolism was present in 45 patients before the institution of treatment, all except 2 recovered.

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Experience with Anticoagulants

By Irving S. Wright, M D

THE GREAT STUDIES of Schmidt, Morawitz, Virchow, Rokitansky and others on the pathogenesis of thrombosis and embolism produced no specific clues leading to a sound method of treatment for these deadly and disabling complications of disease.[1] Just 24 years ago Morawitz made the following statement. "Even today it is still a thankless task to discuss the problem of thrombosis on the sickbed; thrombosis has lost none of its danger; it is still a fearsome disease, a frightening spectre to the surgeon and the physician. We still seek vaguely hither and thither for prophylactic and therapeutic measures." To one working in the field of cardiovascular diseases, the lack of agents with which to combat the thrombosing process was a constant source of frustration. Heparin was theoretically available after McLean's[2] discovery and Howell's early work[3] but actually this was not true because of the difficulty of preparation, the impurity of the product, the severe reactions which forbade its use in man, and the great expense involved in producing small amounts. It was therefore an interesting tool for the laboratory but not safe for man. Best, Scott, and Charles had taken the first major step toward producing a heparin suitable for use in man in 1934, but it was not available for general use for another 5 years. When, therefore, in 1938 I lay in bed for 4 months harassed by a severe thrombophlebitis which occurred after an appendectomy, and which finally burned itself out after producing almost daily fevers of 102 to 103 F and a total loss of 60 pounds in weight, I had both time and special cause for contemplation on this subject. The same year we learned that Best and his co-workers[4,5] had succeeded in producing satisfactory heparin in sufficient quantities so that it could be used in the treatment of thrombosis in man without the risk of severe reaction, provided careful control of the clotting time was observed.

In the fall of 1938 a young man (A. S.), aged 31, was seen in consultation with Drs. Leo Mayer and Jerome Marks. He had been suffering from an intractable and migrating thrombophlebitis which had involved the veins of the legs and the superficial veins of the trunk. In addition, there was clinical evidence of involvement of the mesenteric, splenic, renal, and probably pulmonary venous systems. Available treatment had failed to change the progressive course of this disease. Drs. Best and Murray generously agreed to come to New York, sharing some of their limited supply of heparin, and to help us set up the continuous intravenous infusion with suitable controls of the clotting time. For 16 days and nights during which all concerned were constantly apprehensive, the infusion continued with clotting times being taken at 2 to 4 hour intervals. There were no untoward incidents. The temperature, which had been elevated daily for months, remained normal throughout and the existing lesions subsided. No new lesions developed. This was most encouraging. At the end of that period our supply of heparin ran out and Dr. Best had no more to give us. Unfortunately, shortly thereafter the patient's fever returned and the course of the disease continued uninterrupted until some months later, when he contracted mumps from one of his children, became very ill, and developed a fever that reached 106 F. This fell to normal by crisis, after which the phlebitis disappeared and did not recur for several years. This was never explained, but the experience with heparin was sufficiently interesting for us to use it in additional patients. This was believed to be the first patient treated with the improved [illegible] although a few [illegible] United [illegible] hep[illegible] it in Can[illegible] [illegible]tional sup[illegible] ha[illegible] able to [illegible] patients, [illegible] we [illegible] [illegible]ining con[illegible] p[illegible] diffi[illegible] [illegible] recc[illegible]

From the Department of Med[illegible], Cornell University Medical College, New Y[illegible]

was not very practical for general use. The need for an anticoagulant that could be administered orally and that was not too costly became increasingly apparent.

Meanwhile, Karl Paul Link and his co-workers had been working for some years in an attempt to isolate the compound that was present in spoiled sweet clover. This factor had been recognized by the [redacted] veterinarians, Schofield and Roderick separately, as the cause of a hemorrhagic disease in cattle. The substance responsible for this phenomenon had never been isolated. Link's experiences will be found in his paper in this symposium.

Beginning in 1940, Karl Paul Link and his co-workers began to publish a classical series of papers on this subject and this was capped in April 1941 by the report of the identification and synthesis of the hemorrhagic agent.[6] This was truly exciting and we immediately wrote asking Dr. Link for supplies of this new synthetic agent [redacted] soon as he could spare some. His response was prompt and generous and shortly thereafter we began to receive supplies of the first oral anticoagulant, which was suitable for use in man, Dicumarol. Link had, previous to his publication, supplied some Dicumarol to O O Meyer, J. B. Bingham, and F. J Pohle at the University of Wisconsin where he worked, and to H. R Butt, E. V. Allen and J L. Bollman at the Mayo Clinic On February 27, 1941, Meyer presented the first report on its use in man at the University of Wisconsin[7] In June 1941, Butt, Allen, and Bollman[8] published a preliminary study of its use in dogs and in a series of 6 human beings. In October 1941, we presented our preliminary experiences with its use in 20 human beings

Immediately upon receiving the first shipment of material from Dr. Link, Dr Andrew Prandoni, who was working with me as a research fellow, and I set up a program to test it in man at the Goldwater Memorial Hospital.[9] While recognizing the potential risk as seen in animals, neither of the previous groups had reported any hemorrhagic complications in man We soon encountered some of considerable severity. For example, one patient who leaned out of bed developed a subcutaneous hemorrhage on 1 flank about 8 inches in diameter. Others developed hematuria. This was naturally alarming and both Dr. Prandoni and I lost much sleep over this, but resolved to continue the studies. As it turned out, this was a significant observation, since several pharmaceutical houses were then ready to release large amounts of this substance on the market without adequate understanding or warning regarding the risk and with no detailed knowledge of how to handle such complications if they occurred in man. This would have made it available to physicians without proper training for this form of therapy who would in turn have been dependent on laboratories where the tests for prothrombin time were totally inadequate for measurement of the activity of this potent but potentially dangerous new drug. With the cooperation of the Council of Pharmacy of the American Medical Association and the pharmaceutical houses, the release was delayed until further studies could be carried out. This was actually a matter of more than a year. It is probable that many tragedies were averted by this cautious step . Why did our patients develop hemorrhages whereas the others had not thus far encountered this complication? We finally concluded that this was because we used the Russell Viper Venom technic for our prothrombin tests This was an accepted method at that time but was not sensitive enough to measure early changes in factor VII and prothrombin activity and since the dosage of Dicumarol in man was uncertain, this presented real danger Another disturbing factor was finding that the dosage of Dicumarol could not be determined on the basis of the weight of the patient Lastly, we found that the poorly nourished, often cachectic, patients we worked with did not react to Dicumarol in the same way as the well nourished patients of the Wisconsin and Minnesota groups.

Communication with Drs Meyer and Allen made it possible for us to cross check our results On May 4, 1942, Drs Meyer and Allen and I, each representing our respective teams, presented data before the American Society for Clinical Investigation and this

was repeated in greater detail before the Section on Experimental Medicine and Therapeutics of the American Medical Association in June 1942.[10-12] The experiences were so similar and encouraging that coming from 3 separate institutions the impact was such as to stimulate others to initiate broader studies. We had an effective anticlotting agent which could be administered by mouth, but now the challenge was to determine the indications and contraindications for its use in the care of patients. The experience in numerous hospitals with its use for the prevention and treatment of thrombophlebitis and pulmonary embolism was rapidly and favorably developed. In May 1942 we started to use it cautiously in patients with heart disease, first for myocardial infarction with embolization, then with rheumatic heart disease with embolization. The patients tolerated the drug well and the clinical impression was encouraging but the material was limited because of the lack of confidence in this form of therapy by others as well as ourselves.

World War II then entered into the picture and undoubtedly delayed the development and acceptance of anticoagulant therapy, since many who were active in this field entered the armed forces. Although we could not obtain official approval from the Surgeon General's Office to stock Dicumarol in Army pharmacies until later, we did succeed in getting tacit permission to continue our studies first at the Army and Navy General Hospital in Hot Springs, Arkansas, and later in numerous army hospitals in the Midwest and and Far West where I served as consultant. Dr. Prandoni also continued to increase his experience with anticoagulants on the Medical Service at the Walter Reed Hospital.

By 1945 we had accumulated data based on the treatment of 76 patients suffering from acute or recurrent myocardial infarction, and this was reported before the California Heart Association on October 18, 1945.[13, 14]

Meanwhile, E S Nichol and S W Page of Miami, and H R Peters, J R Guyther, and C E Brambel of Baltimore had been accumulating series of patients suffering from myocardial infarction treated with Dicumarol Their experiences were published early in 1946 and were in agreement with ours[15, 16] This was encouraging but the data were not conclusive and we therefore proposed the cooperative study which was carried out by the Committee on Anticoagulants of the American Heart Association. The report of this committee includes the details of its work. This large project enlisted the resources of 16 leading medical institutions with teams of workers each headed by an outstanding cardiologist. A central laboratory and statistical center at the New York Hospital acted as the coordinating agency. Cases admitted on alternate days were admitted to treated and controlled series Master forms were compiled in detail and analyzed by Dr. Dorothy Beck, Chief Statistician, and her staff. In 2 years the case records of 1,031 patients were secured. Preliminary reports were issued, but the final report took more than 6 years to complete.[17, 18] Following the publication of this report the use of anticoagulants for the treatment of myocardial infarction was adopted widely in many countries as well as in the United States Although there still exist some differences of opinion regarding the selection of suitable cases, there have been more than 60 confirmatory reports published from medical centers in this country and abroad and the wide use of this form of therapy seems to be accepted for the foreseeable future.

There are now many anticoagulants of the coumarin and phenylindandione groups available. We have evaluated a number of them. They vary somewhat in onset and duration of action but present few advantages over Dicumarol and phenylindandione as they were first made available for general use

As indicated above, in 1942 we began the treatment of patients with multiple embolization from old rheumatic heart disease and myocardial infarction Thus evolved the conception of long-term anticoagulant therapy. Some of these patients had developed cerebral emboli and it seemed logical to attempt to interrupt a tragic series of events leading to death or perhaps even worse, complete invalidism. From these experiences we were encouraged to treat patients with cerebral

thrombosis including carotid artery and basilar artery thrombosis This was seriously embarked upon in December 1946 and the results have been reported at intervals since that time[19] This work has expanded and is now being submitted to analysis in several cooperative long-term studies in which our group is actively participating. Great credit is due to Dr. William T. Foley and Dr. Ellen McDevitt for their consistent work in this field during the past decade[20-23] The final evaluation of the indications and contraindications for the use of anticoagulants in the treatment of cerebral thrombosis remains to be concluded during the coming years.

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Historical Data Regarding the Experiences with Coumarin Anticoagulants at the University of Wisconsin Medical School

By Ovid O. Meyer, M.D.

IT WAS 1933, 20 years ago, when, in conversation with Professor R. A. Brink of the Department of Genetics at the University of Wisconsin, that I first heard of the work being done in the Department of Agriculture to identify the substance in spoiled sweet clover accountable for hemorrhagic disease in cattle. This had been initially undertaken in 1934 at the Wisconsin Agricultural Experiment Station by Professor Karl Paul Link and his associates. This group of workers, including Campbell,[1] Stahmann and Huebner,[2] finally isolated the anticoagulant, a coumarin derivative, and established its chemical characteristics. The yield was about 1 Gm per ton of clover, an amount which was not practical for clinical usage However, by April 1940, these investigators had synthesized a 3-substituted-4-hydroxycoumarin which was chemically, physically, and biologically identical to the naturally occurring substance. This could be prepared cheaply and in abundant quantities The potential significance in the treatment of thromboembolic disease was then obvious, and hence we were happy when a supply was made available to us for basic studies in September 1940 The anticoagulant for clinical purposes was given the name Dicumarol.

My early associates in the field included James B. Bingham, now in Seattle, Dr Frederick J Pohle, deceased, Dr John McCarter, now of Boise, Idaho, Dr Charles T Thill of Chicago, and Dr Marylou Spooner Schallek of Nutley, N. J It was promptly established that this anticoagulant was a potent hypoprothrombinemic agent in vivo in dogs and without effect in vitro. Our original experiments were set up to elucidate the morphologic changes that might occur in dogs when so-called therapeutic and toxic doses were given, to establish the range between the effective therapeutic dose and the minimal lethal dose, to determine whether or not this anticoagulant reduced the prothrombin in human beings [illegible] it had been shown to do in cattle, rabbits, rats, mice, guinea pigs, and dogs, and to demonstrate whether administration of this dicoumarin in safe dosage would actually prevent or prolong the time of intravascular clotting The final and most important objective, of course, was to establish whether or not the anticoagulant would prevent the development of thromboses in human beings investigation of the above effects was mainly directed toward this major aim, which obviously could be settled "only by extensive investigations of the future."[3]

Our first published report[3] established that the administration of oral (the powdered substance given in a gelatin capsule)* or intravenous administration of Dicumarol produced, after a usual latent period of 24 hours, prolongation of the prothrombin time (and coagulation time if measured at room temperature but not if properly measured in a water bath at 37-38 C) It was further demonstrated that therapeutic and even fatal doses did not produce significant pathologic changes in the liver or other parenchymal organs It was shown[3,4] in dogs, however, that excessive or fatal, but not therapeutic, doses produced

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*3,3' methylenebis (4 hydroxycoumarin) is highly insoluble in water. At a pH of 10 or more the sodium salts are soluble but the solutions are unstable and must be used promptly after being prepared

hemorrhages, gross or microscopic, toxic lesions, and marked dilatation of small arteries, arterioles, capillaries, venules, and small veins, acute renal glomerular swelling, and toxic lymphoid degeneration These effects are emphasized, since several authors, at least by implications, have indicated that Dicumarol is a vasodilator, which it is not unless toxic doses are administered We have never observed vasodilatation when proper doses are employed

After gathering, with a less than ideal method, some suggestive experimental evidence that Dicumarol did prevent some intravascular (jugular) clotting in dogs[3] and after establishing the safety of the drug in animals and an approximately proper dosage, we cautiously administered it to human beings to achieve the data needed for clinical use. The first 12 patients who received the drug were, with 1 exception, patients with advanced malignancy. The patients, save 1, demonstrated no definite clinical evidence of involvement of the liver. The very first patient received a total dose of 10 mg. and the other 11 patients received doses of 50 to 100 mg (0 75 to 2 0 mg per Kg). In 5 of these 11 the prothrombin time was slightly prolonged. It was then promptly established in additional patients that a dose of 4 mg per Kg. was safe and could produce significant hypoprothrombinemia Still later[5] it was found that, in our hands, the most satisfactory therapy entailed the use of an initial oral dose of 5 mg. per Kg , followed by doses of 1 5 mg. per Kg , on those days when a dose could be given to maintain the prothrombin at 50 to 25 per cent. As is well known, subsequent studies by many others as well as ourselves have established that still lower levels of prothrombin are better.

The first public report of our experiences with this anticoagulant was made in the discussion of a paper presented by Professor Link before the University of Wisconsin Medical School Society, February 27, 1941.

By early winter, 1942, we had more clearly demonstrated, as had others,[6] the permissibility of giving the anticoagulant for as long as 5 weeks without toxic effect and the absolute need for daily or at least frequent prothrombin time determinations if the drug was to be safely administered. We found that blood transfusions appeared to be effective in controlling excessive hypoprothrombinemia and that vitamin K was ineffective, but we used doses no larger than 10 mg given orally or intramuscularly. However, Shapiro et al.[7] demonstrated that vitamin K in relatively large amounts did counteract Dicumarol-induced hypoprothrombinemia and Cromer and Barker[8] found that large doses, 64 mg. of menadione bisulfite intravenously, usually were effective in correcting excessive hypoprothrombinemia due to administration of Dicumarol.

In 1943 we observed[9] that the rectal administration of Dicumarol in suppositories was not regularly effective. Miss Maryloo Spooner, while a graduate student at the University of Wisconsin, established, using the method of Helen Wright,[10] that Dicumarol decreased the adhesiveness of platelets without affecting the platelet count per se

By 1943 there were several workers investigating coumarin anticoagulant therapy, and the practicality and usefulness of this drug became increasingly evident. It has been widely established that the only hazardous effect of this treatment was hemorrhage, and this was likely, of course, in patients who had ulcerative lesions, a hemorrhagic tendency significant liver disease, or in the rare patient who was unusually sensitive to the drug in ordinary dosage. Some rare individuals were unusually resistant and required large doses However, we sought a more ideal anticoagulant, a fixed dose of which would produce a fixed proportional hypoprothrombinemic effect, in order to avoid, insofar as possible, the need for frequent, troublesome, costly, and sometimes unavailable prothrombin time determinations

Hence we were pleased to test another coumarin compound, 4-hydroxycoumarin no 63, made available to us by Professor Link and Dr Lester D Scheel in May 1949. This synthetic chemical, 2-methyl-2-methoxy-4-phenyl-5-orodihydropyrano-(3-2c)(1) benzopyran,

was first tested in dogs, a dose was established, and it was later tested in human beings.[11] The results indicated that this anticoagulant was 2 to 3 times as potent as Dicumarol and that lethal doses in dogs did not produce the toxic lesions in the small blood vessels that occurred with excessive administration of Dicumarol. Although it appeared from these and later studies[12] that a greater stability of hypoprothrombinemic maintenance seemed possible with this drug (cyclocumarol) and that it had no apparent disadvantages, it never became popular, and in our own clinical work it was subsequently replaced when warfarin sodium was introduced. While testing this latter anticoagulant, we first observed that vitamin K_1 and vitamin K_1 oxide were very effective antidotes for excessive hypoprothrombinemia due to this and the other coumarin anticoagulants. James et al.[13] had previously reported this observation for vitamin K_1 oxide. Obviously this was an important addition to our armamentarium, since it appreciably lessened the hazard of coumarin anticoagulant treatment.

While testing this anticoagulant, we inadvertently learned of another hazard in this type of therapy.[14] In retrospect, it seems humorous, but it was not funny at the time and it might have been tragic. Two patients of the same name were on the same hospital ward. One was receiving the 4-hydroxycoumarin anticoagulant, and the prothrombin time determinations were carried out daily on the blood of the other. Since the former patient appeared to be resistant to this drug and the daily prothrombin of the latter was 100 per cent, the dosage was progressively increased, and only when subcutaneous bleeding developed at the site of hypodermoclysis needle punctures in the patient receiving the anticoagulant was the error realized and corrective measures taken. This was the fifth of 200 patients who demonstrated gross hemorrhagic side effects attributable to cyclocumarol.

The ideal anticoagulant had still not been discovered, nor has it yet. The toxicity of those available was low, and effectiveness was demonstrated. Nevertheless, it was still hoped that an anticoagulant with more regular response in prothrombin reduction to any given dose and with greater stability of levels of prothrombin might become available so that less frequent prothrombin determinations might be possible.

Synthesis of the 3-substituted-4-hydroxycoumarin anticoagulant no. 42, 3-(α phenyl-β-acetylethyl)-4-hydroxycoumarin, was first accomplished and its action studied in Professor Karl Paul Link's laboratory.[15, 16] This compound was named warfarin, and its readily water-soluble sodium salt was warfarin sodium. This anticoagulant was first kindly supplied by Professor Link and later by Dr. Samuel B. Gordon of Endo Products, Inc., Richmond Hill, N. Y. Initially in 1953 we used both warfarin and warfarin sodium, which are more potent than the other 2 anticoagulants. Later, since the former had no advantages, we continued our studies with only warfarin sodium, which we have found subsequently can be used intravenously and intramuscularly with safety, as well as orally.[17] To my knowledge, no other coumarin anticoagulant can be satisfactorily employed parenterally. In 1956[18] we found that, unlike Dicumarol, warfarin sodium was consistently effective when administered rectally. Our investigations demonstrated that this anticoagulant was superior, not only because it could be given parenterally if the need existed, but because the latent period, which was the same for oral and parenteral administration, was shorter than for either Dicumarol or cyclocumarol (Cumopyran). Even more important, with warfarin and warfarin sodium it has been easier to maintain the prothrombin level steadily within the therapeutic range. Hence, the staff throughout our hospital has found it generally easier to manage the patients requiring anticoagulant therapy. The hazards of anticoagulant therapy are the same for warfarin sodium as for other coumarin compounds, though perhaps somewhat lessened, and vitamin K_1 has been found to be a satisfactory antidote for the excessive hypoprothrombinemia which could result. In our

institution for the past 3 years Dicumarol and cyclocumarol have been almost entirely superseded by this newer coumarin compound

This concludes my remarks, limited to the contribution of the investigators at the University of Wisconsin Medical School. These historical facts are related as accurately as possible in order to make our segment in the final historical profile complete and graphic. The total picture will point out the numerous contributions of many investigators in the elucidation of this very interesting subject. Once more there is emphasized the importance of fundamental progress from the laboratory of the scientist making the original discovery to the final successful clinical application. Obviously many unanswered problems remain in this field, and there is much opportunity for other workers to perfect the applications of the present information and to augment these facts with additional, much needed information

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My Early Experience with Bishydroxycoumarin (Dicumarol)

By Edgar V. Allen, M.D.

" . . . leaven to quicken the blood that ran . . . "
A. C. Swinburne, 1878

IN THE LAST of March 1941, my associate at the Mayo Clinic, Dr. H. R. Butt, gave a lecture at the weekly meeting of the staff of the Mayo Clinic on the use of vitamin K to correct deficiency of prothrombin associated with jaundice and disease of the liver. In the course of that lecture he mentioned a compound that had been prepared which was capable of producing a deficiency of prothrombin in animals. This state apparently could not be corrected by the administration of vitamin K. After the lecture I asked Dr. Butt about the new preparation, and found that he had read about it only a few days previously in the *Journal of Biological Chemistry*. The work had been done by Dr. Karl P. Link and his associates at the University of Wisconsin. Dr. Butt had been working with vitamin K and measurements of prothrombin for several years, and he was greatly interested in this new discovery

A request was sent to Dr. Link for a supply of some material for clinical use. We discussed at some length the possible clinical uses of this material but, to the best of our knowledge, it never had been administered to a human being, so all we could do was to speculate. We did have high hopes Dr Link and his associates very promptly sent to us some bishydroxycoumarin (Dicumarol) A study of the effect of this compound on dogs was begun by Dr J L Bollman about the middle of April 1941 The results of his studies confirmed the observations of Link and his associates

My associates and I who were interested in intravascular coagulation had used heparin for a number of years Heparin was known to be valuable in the treatment of vascular thrombosis and embolism, but it had disadvantages, specifically in its short action, its need for parenteral administration, and its considerable cost. I had believed for some time that another preparation could be used that would abolish these objections and tha' would be beneficial in the care of patient with vascular thrombosis and embolism

On May 9, 1941, Dr. Butt and I administe ed Dicumarol to an organically sound young man who was 19 years old and who weighed 80 Kg. We had no alternative but to guess at the proper dose; we gave too much, that is, 1.8 Gm. in 5 days. On the sixth day after our patient first swallowed the Dicumarol, Miss Margaret M. Hurn, who was determining prothrombin activity in the blood, called me, with some concern, to say that our patient had almost no prothrombin in his blood (fig 1). Moreover, the coagulation time of the blood was prolonged (fig. 2). Dr. Butt and I shared Miss Hurn's concern, for we were conscious of the possibility of severe hemorrhage

Although at that time vitamin K was considered to lack the ability to increase prothrombin activity when deficiency of prothrombin was induced by Dicumarol, we gave the patient 20 mg of synthetic vitamin K intravenously, at the suggestion of Dr. Butt The prothrombin time decreased from 140 to 87 seconds within 24 hours, but at the end of another 24 hour period the prothrombin time was 161 seconds It was only many months later, when reviewing the clinical record of the patient, that we recognized that there had been substantial increase in prothrombin activity attributable to the use of vitamin K Until that time we had believed that the recorded change in prothrombin activity had been a "normal fluctuation" When we finally recognized, in retrospect, the specific effect of vitamin K on the blood of our patient, it had already been demonstrated that large doses of

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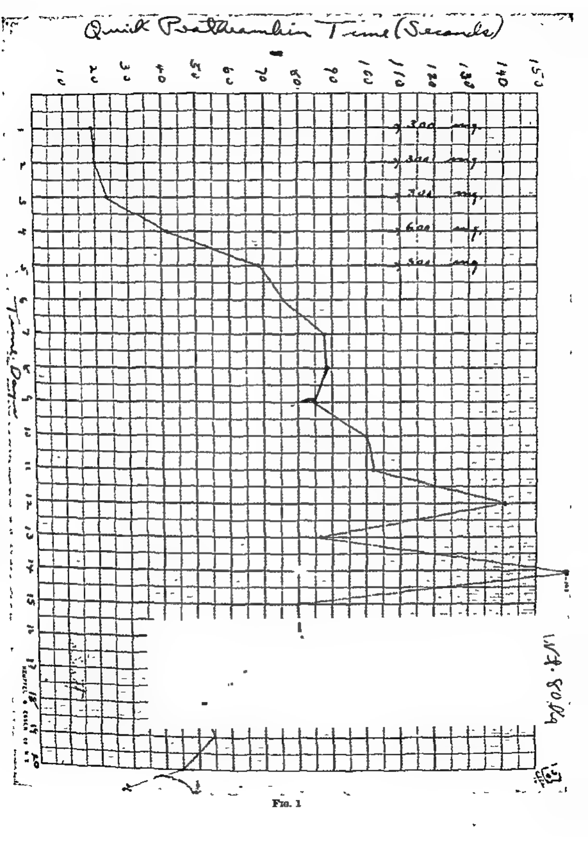
Quick Prothrombin Time (Seconds)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
300 mg.
300 mg.
300 mg.
600 mg.
300 mg.
Time, Days

Fig. 1

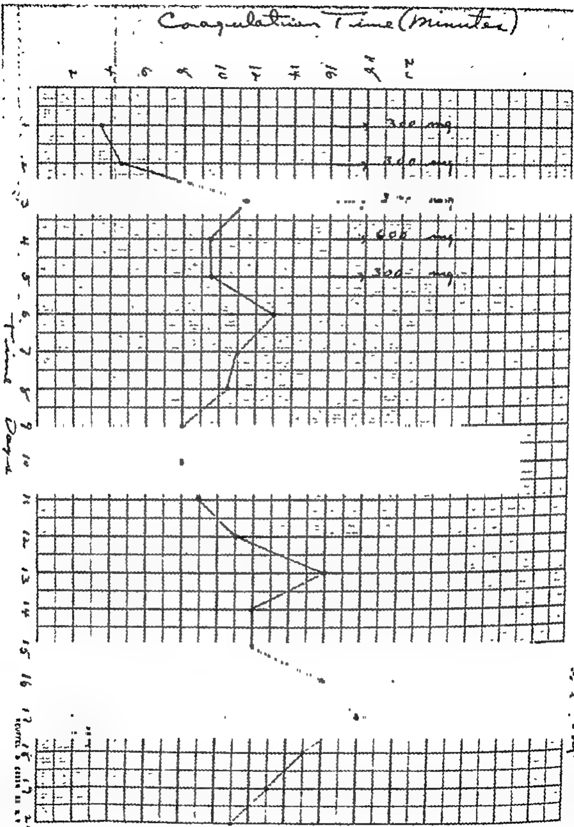

FIG 2

vitamin K enhance prothrombin activity when deficiency of prothrombin has been induced by the use of Dicumarol, although small doses fail to do so.

We protected our first patient from injury, and within 20 days the prothrombin activity and the coagulation time of the blood had returned to normal (figs. 1 and 2). His health had not been impaired in any way.

At that time Dr. Butt and I believed that we were the first to administer what since became known as Dicumarol to a human being, but Dr. Link said in his Harveian Lecture, delivered on January 20, 1944, that the anticoagulant had been given to the clinical group at the Wisconsin General Hospital, Madison, in September 1940. Dr. Ovid O. Meyer subsequently told me that he and his associates had first given Dicumarol to human patients in December 1940 or in January 1941. However, the first patient received 10 mg. of Dicumarol on the first, third, and fifth days, 25 mg on the sixth day, and 50 mg. on the tenth day of observation. Eleven additional patients, with one exception, received single doses ranging from 50 to 100 mg. of Dicumarol Study of the blood of these patients showed that in 5 instances there was prolongation of the prothrombin time from an average control level of 10 seconds to an average maximal level of 12 seconds

When I was attending the annual meeting of the American Medical Association in 1941 I received a telegram from Dr. Butt relative to some aspect of the problem Shortly afterward I told Dr. Irving S. Wright, of New York City, that we were carrying out studies that might have great importance. I told him I did not wish to disclose the nature of the studies because they were in a preliminary stage. He replied that he also was anticipating carrying out studies of interest, but declined to give information for the same reason I had declined. It was not until some time later that it was disclosed that both of us had Dicumarol in mind.

In the issue of the *Proceedings of the Staff Meetings of the Mayo Clinic* for June 18, 1941, Dr. Butt, Dr. Bollman, and I published the first report of the administration of Dicumarol to man.

Of this report Dr. Link wrote in his Harveian Lecture, "Boldness, a combination of right talent and an objective point of view . . . enabled them to publish the first preliminary clinical report . . . ," and that "Messrs Butt and Allen gave it in full therapeutic doses to six human subjects."

The editorial comment on our report in the September 13, 1941, issue of the *Lancet* was given the title, "Heparin and a Rival." On May 4, 1942, the presentations on behalf of the speakers and their associates were by Dr. Irving S. Wright, of New York City, Dr Ovid O. Meyer, of Madison, Wisconsin, and by myself before the American Society of Clinical Investigation At the annual meeting of the American Medical Association in June 1942, 3 more detailed presentations were made before the meeting of the Section on Experimental Pharmacology and Therapeutics.

Needless to say, Dicumarol and similar substances have proved of great value in clinical practice; the use of them has saved many lives The story which I have related, based on my personal participation in it, is the story, "From the Haystack to the Human,"* for treatment of diseases of man with Dicumarol resulted from original studies by Link and his associates on the cause of hemorrhagic disease of cattle which occurred when they ate spoiled sweet-clover hay.

There is another chapter yet to be written Identical amounts of Dicumarol and substances that have a similar action produce diverse effects on the prothrombin activity of different persons, and on the prothrombin activity of the same person at different times Hence, an effect that is constant in quality cannot be anticipated accurately What is urgently needed is a preparation which, when administered in the same amount, always produces the same effect Such an accomplishment seems improbable and perhaps impossible, but we are bound to remember that in 1939 Dicumarol also was "impossible"

*The designation of a newspaper reporter.

Historical Notes on the Early Development of Anticoagulant Therapy with Dicumarol in Sweden

By JÖRGEN LEHMANN, M.D., PH.D.

THE INTEREST in anticoagulant therapy of thromboembolic diseases was stimulated in Sweden during the years 1935 to 1937 due to the work of Jorpes and co-workers on heparin. The author's participation in this field was facilitated when he was appointed in 1938 as director of a newly erected central laboratory for clinical chemistry in the city hospital of Gothenburg-Sahlgrens Hospital. A new laboratory building, equipped with animal rooms, operating room, etc for work in experimental medicine, was opened on September 1, 1940 A few days before this celebration, a big truck turned up at the front side of the laboratory building, loaded with hay of sweet clover (*Melilotus albus*) harvested at the dustyard at Hisingen, outside Gothenburg At the top of this load the assistant doctor of the laboratory, Johan Mårtensson, was seated, and the truck was followed by the author, riding a bicycle Astonished and wondering laboratory technicians gathered at the windows at this peculiar appearance—this was something unusual

The hay was brought into a small, closed room of the laboratory and left for spontaneous molding, after which it was fed to rabbits The aim was to see if the hemorrhagic disease described in cattle by Schofield[1] and Roderick,[2] mentioned in a paper by Quick,[3] could be reproduced This would be of interest, as the toxic principle in the hay might be extracted and purified for clinical use in thromboembolic diseases It was suspected to be a derivative of coumarin This anticipation was based on a personal communication during the summer of 1940 from Dr Gote Turesson, Professor of Plant Systematics and Genetics at the Royal Agriculture College in Upsala, whom the author had told about the bleeding disease in cattle. Dr. Turesson mentioned the high concentration of coumarin in sweet clover and the efforts in Canada of producing new coumarin strains which might produce a better food for the cattle. We looked up the structural formula of coumarin, and the author was struck by the close relationship between coumarin and naphthoquinone, the essential part of the recently synthesized vitamin K (Fieser[4] and Doisy[5]). This relationship might possibly explain the mechanism by which the toxic principle was active, namely, as a competitive inhibitor of vitamin K, thereby depressing the synthesis of prothrombin in the liver and producing a lowered coagulability of the blood.

The author was familiar with such substrate inhibitions of enzyme activity from many years of work on dehydrogenases in Thunberg's laboratory at the University of Lund, and especially from the work of Quastel and Wheatley[6] on the specific inhibition of the oxidation of succinic acid by the closely related malonic acid.

The relationship between coumarin and vitamin K brought the sweet clover experiments in touch with earlier experiments of the same year (1940) in which preliminary experiments with naphthoquinone as a vitamin K inhibitor had been performed—but in vain

Other methods of producing inhibition of the coagulation process were then tried (July 1940) with Benzoechtrosa (Kahlson and Landby[7]) but were found to be not applicable in the clinic

After the visit to Dr Turesson in Upsala the author's interest was directed toward coumarin Even before the experiments with molded sweet clover were started, coumarin was tried as an anticoagulant in rabbits, given intramuscularly in doses of 0 15 to 0 20 Gm, dissolved in sesame oil, and 0 5 Gm dissolved in 6 ml of 30 to 45 per cent (v/v) ethanol

From the Central Laboratory for Clinical Chemistry, Sahlgrens Hospital, Gothenburg, Sweden

and given by stomach tube. With the last doses definite prolongation of the prothrombin time, but not of the spontaneous coagulation time, was achieved (August 15 to 21, 1940). These experiments were considered as unpromising and given up.

Experiments with spontaneously molded sweet clover hay were begun in rabbits (October 11, 1940). No fully conclusive results were obtained. The experiments were continued in 1941, when the hay was molded with *Aspergillus niger* and *Aspergillus fumigatus*, kindly supplied by Dr. Rennerfeldt at the Botanical Institute of Gothenburg and known to be active in producing the "toxic" substance. On March 23, 1941, a successful series of experiments was started, succeeded by preliminary extraction experiments. However, the papers of Dr. K. P. Link and his coworkers[8-11] on the isolation and synthesis of the active principle in spoiled sweet clover hay appeared in the Journal of Biological Chemistry, and these experiments were therefore stopped (May 1941).

A new epoch opened for us when 3,3'-methylene-bis (4-hydroxy-coumarin) was synthesized by Mr. Rosdal at the Ferrosan Company, Malmö, Sweden. The first sample was received on June 26, 1941. Animal experiments began on June 30 in 13 rabbits and a few dogs and were finished on August 14. The reversibility of the prolongation of the prothrombin time and coagulation time was demonstrated. No liver injury could be demonstrated by microscopic examination even after long-term treatment of the animals. The antagonistic effect of blood transfusion was shown. Synthetic vitamin K was also tried in a dose of 5 mg to a rabbit but did not inhibit the effect of Dicumarol (July 12, 1941). This was a great disappointment, as the anticipated competitive inhibition of vitamin K was thereby questioned. However, later in a critical situation with an oozing bleeding from the intestinal mucosa of the anus in a young woman, 200 mg of vitamin K (2 methyl-1,4-naphthohydroquinone disulfate) was given with immediate effect (Jan 5, 1942, Surg. Dep. II, Jl Nr 3347/41).

When the animal experiments were finished, the author was very much in doubt if they should be published at once. In spite of the fact that they were promising as an anticoagulant treatment for thrombotic diseases, which was the aim of the experiments, it was decided not to publish any experiments before the effect had been demonstrated in patients suffering from thromboembolic disease. The main argument for this decision was the critical attitude which clinicians in Scandinavia often had shown against animal experiments as a guide to human therapy. Premature conclusions would presumably hurt the future development of the experiments. The author was even cautious in keeping the experiments secret within the laboratory as well as within the hospital. In the animal protocols Dicumarol was signed as "X-substance."

The details of the early clinical use of Dicumarol in Gothenburg have been published elsewhere.[12-14] The care of the patients with thrombosis during the first years was assigned to the author, who is especially grateful to Dr. Gustaf Pettersson, Surgical Department II, the first doctor in the hospital to whom the Dicumarol experiments were mentioned, and whose patients were the first to be treated. (The first patient suffering from thrombosis was treated on October 11, 1941. Surg. Dep. II, Jl Nr 2619/41.)

After the first presentation of the clinical results November 29, 1941, in Stockholm (published in *Svenska Läkartidningen*, January 9, 1942), the author was confronted with the problem of how to get a paper published in the international literature. All regular communications with England and America had stopped because of the war. The only communications with England were irregular and by planes during the night. These were often heavily attacked by German planes. However, the British Consul General in Gothenburg was kind enough to take care of a paper for *Lancet* (December 23, 1941).

Nothing was learned about the fate of the paper until a letter arrived from Dr. Link,

dated April 20, 1942 With Dr. Link's permission the letter is here published.

Dear Dr. Lehmann,

I was very glad to see your account in the Lancet entitled Hypo-prothrombinaemia produced by Methylene-Bis-(Hydroxycoumarin) 3/14/42.

It is clear from this excellent and highly condensed note that you have made very substantial progress toward evaluating the possible therapeutic potentialities of the substance. I would be very glad to have reprints of your work as it appears.

By separate post I am sending you reprints of our work which has appeared in print to date. The following papers are in press:

VIII. The effect of 2-Mthyl-1,4-Naphthaquinone and 1-ascorbic acid upon the action of 3,3'-methylene-bis (4-hydroxycoumarin) on the Prothrombin Time of Rabbits Jour. Biol. Chem.

IX. The effect of diet and Vitamin K on the Hypoprothrombinaemia induced by 3,3'-methylene-bis (4-hydroxycoumarin) in the rat.

A critical study on the role of l-ascorbic acid in the rat and guinea pig in so far as it affects the dicoumarin has been completed and will be ready shortly.

We were very much impressed by the fact that your reasoning on the action of l-ascorbic acid and the possible antagonistic action of vitamin K paralleled our thinking. Furthermore I think in figure 1 of paper VII and the first figure in your Lancet article bear a striking parallelism

With best wishes and kindest regards,

Karl Paul Link

None of the papers from Dr Link was ever received From the author's answer to Dr. Link, dated July 13, 1942, the following is quoted

Dear Dr. Link,

Very many thanks for your kind letter of 4/20/42, which I received 6/25/42 It was the first announcement to me, that my paper had been printed in the Lancet. I sent it to the editor 12/23/41 and have not heard anything about the fate of the paper until I received your letter. We have only air mail connection with England and the sendings are therefore very restricted. We can't get the Lancet here in Sweden Therefore, Dr. L... letter was especially welcome I have... the editor for a few reprints and I w... one as soon as I have got it It c... summary of my other papers, which ... in Swedish It will be of great ... to read the papers mentioned in your letter. They have not arrived yet.

An attempt was even made in April 194' to have a paper published in *Science* in th U.S.A. as elucidated from the following lett to a friend of the author, Dr. Frederick Ber heim, Duke University School of Medicine.

Goteborg, April 11th, 1941

Dear Frederick,

A friend of mine, captain on a Swedish boat,* has promised me to forward this letter to you, when coming to your continent. I enclose parts of a paper, which I think will be of interest for you. When I had finished the paper in 1941 I had written a summary for Science, but just as I was going to send it, the post for U.S.A. was stopped If it is possible I should be glad if you would forward it to Science. For such a case, please correct it and make a choice of the figures. I am sorry not to be able to send the figures to paper 2 (in print), but I think it can be printed without the figures. I have heard of a friend of mine, that some doctors at the Mayo clinic (H. R. Butt, E. V. Allen a. J. L. Bollman) have been working on the same subject. Would you kindly send them paper II when having used it for Science. I send them the summary of paper I. (They are working at the Mayo clinic, Rochester, Minn.)

Even the fate of this paper was unknown to the author for about a year. From a letter to Dr Bernheim February 28, 1943, it is evident how the author received information about its publication October 9, 1942

Dear Frederick,

Very many thanks for your letter of November 3, 1942, which I received February 26, 1943. For a week ago I heard from the Swedish-American News Information ... 'm that a pa... mine in Science ... tioned in ... News Letter and ... nderstood th... had got my ... to ... about it to ... glad ... kindness.

The poor co... burg and U.S.A.

*The boat Stec... and the captair...

the following letter, dated June 1, 1943, to Dr Bernheim.

Dear Frederick,

. . . Further I should be glad to know if any papers have appeared in U.S.A on the use of the dicoumarin in thromboembolic diseases and their results In the Lancet May 15, 1943 my clinical results for 1942 have been published. The dicoumarin is now in use in many hospitals in Sweden and so far I know with good results.

The last phase in the development of the use of Dicumarol was the treatment of coronary infarction. The first case, complicated with a thrombosis antecruris, was treated November 7, 1941, (Dep Vasa. . . . Julia M-g, admitted November 2, 1941). On proposal of, and in cooperation with, Dr. Bo Ewert, Medical Department I, a more consistent but cautious treatment of selected cases was started in 1942 Of 47 cases, 16 were treated with 0 per cent mortality as compared with 45 1 per cent of the untreated group. During 1943 the corresponding figures were 31, of which 16 were treated, with 25 per cent mortality as compared with 64 2 per cent in the untreated group During 1944, 57 cases were treated of a total of 76, with 25.9 per cent mortality as compared with 47.3 in the untreated group, and in 1945 (January 1 to June 30) 43 out of 46 were treated, with a mortality of 20 9 per cent Since then nearly all cases have been treated with Dicumarol, often combined with heparin (The figures mentioned above have been compiled by Dr Albert Larsson, and read before the Swedish Society for Internal Medicine, September 8, 1945, but this paper has as yet not been published)[17]

After 1945 the treatment of thromboembolic patients as well as of patients with coronary infarction in Sahlgrens Hospital was taken over by the physicians of the different departments In many hospitals in Sweden Dicumarol was then in use. Especially at the University clinic in Lund a careful study was made of the prophylactic and curative use of Dicumarol in surgical patients.[18]

This review of the early events in the use of Dicumarol in Sweden can best be finished by quoting a letter from the author to Dr. Link dated June 13, 1942

It was surprising but a striking fact, that the year 1941 was mature for a more detailed study of the toxic agent in sweet clover hay as it was studied at the same time in your laboratory and here. I have now treated nearly 200 patients with the dicoumarin and the results are even as good as those from patients treated with heparin.

I hope I will be able to meet you once in the future, when the world has found itself again. I was working 14 months 1935-1936 in the Rockefeller Institute in New York (Neuro-physiology by Dr. Gasser) where I spent some of the most interesting time in my life and I do hope I will get time for another trip to U.S.A.

With kindest regards,

Sincerely yours,

Jorgen Lehmann

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Post-Thrombotic Sequelae Preventable with Anticoagulant Therapy

By Harry Zilliacus, M.D.

AFTER FIVE YEARS of duty as a military physician in the war, I felt a strong urge to carry on some research investigation. As conditions for this were very poor in Finland at that time, I left for Stockholm, where my former teacher of physiology, Prof. R Granit, working at the Karolinska Institutet, did me the great favor of introducing me to Prof Erik Jorpes in December 1944 By that time Professor Jorpes was well along in the development of the concept that thromboembolism could be controlled in large measure by the use of anticoagulants Favorable results for prophylaxis and treatment with heparin had been reported in Sweden by Jorpes, Crafoord, Watterdal, Bauer, Simon, Suve, and Linde On the other side of the Atlantic encouraging results were reported by Best, Murray, and McKenzie Similar advantages had been reported following the use of Dicumarol by Wright, Prandoni, Meyer, Allen, Barker, Nygaard, Walters, Priestly, Waugh, Butsch, and Stewart, in the United States, and by Lehmann and Bruzelius in Sweden

In 1945, Bauer, reporting a series of 103 patients, drew attention to the possibility of preventing post-thrombotic sequelae by treating the very early stages of deep venous thrombosis with heparin. As these observations were based on relatively few patients there remained some doubt about the specificity and effectiveness of the anticoagulants I felt, therefore, very fortunate to be asked by Professor Jorpes to organize a study, based on a large number of patients, to evaluate anticoagulant therapy in the control of acute deep vein thrombosis and reduction of the risk of pulmonary emboli and other post-thrombotic sequelae Through the courtesy of the Chiefs of Staff of 15 leading hospitals in Sweden and 2 in Finland it was possible to review the material on 1,158 cases of deep thrombosis of the veins of the legs. This report was published under the title, "On the specific treatment of thrombosis and pulmonary embolism with anticoagulants, with particular reference to the post-thrombotic sequelae" (*Supplementum Acta Medica Scandinavia*, 1946). It was found that specific therapy with anticoagulants shortened the time the patient had to stay in bed to about one fifth of that required by conservative therapy The thrombotic process could usually be limited to the vein in which it was first diagnosed It was also established that under anticoagulant therapy the frequency of pulmonary embolism in thrombotic cases decreased from approximately 30 to 0 5 per cent, and that the earlier high mortality from this much dreaded complication was reduced to almost nil In the course of follow-up examinations of 609 patients who had suffered from an acute deep venous thrombosis from 1 to 5 years previously, it was found that post-thrombotic sequelae, including chronic edema, induration, eczema, pain, and leg ulcer, occurred in 4 out of 5 patients conservatively treated In those patients in whom early diagnosis was still limited to the calf, and anticoagulants were administered, post-thrombotic symptoms were mostly absent or very mild One decade later, in 1946, Gjores (Acta Chirurgica Scandinavia, Suppl 206) found in a follow-up study of 303 patients an incidence of post-thrombotic sequelae very similar to the corresponding figures in my investigation

In the discussion of my thesis I had stressed that thrombosis, probably due to anatomic reasons, was found much more frequently in the left than in the right leg, and that this was reflected in the more frequent occurrence of post-thrombotic sequelae in the left leg. I

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had pointed out that this correlation could be confirmed by watching ladies legs in the street, and soon thereafter I received a telegram in which congratulations were sent by The Society for Saving the Beauty of Ladies' Legs. I have always suspected that this originated from Professor Jorpes!

The great number of thrombotic cases examined in the course of this investigation established the fact that anticoagulant therapy for thromboembolism was about as specific as insulin therapy for diabetes The evidence clearly demonstrated that with the aid of anticoagulants thrombosis could be controlled in the acute stage, and post-thrombotic sequelae thus avoided. My additional studies in this field are found in the reference list of this article.

This early experience with the problems of coagulation and thrombosis has been of the greatest value in the prevention and treatment of thrombosis on my obstetrical service, where 5,000 obstetrical and 1,000 gynecological patients enter the I. University Hospital for Women in Helsinki annually.

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Personal Experiences with Anticoagulants for Coronary Atherosclerosis

By E. Sterling Nichol, M.D.

CLINICAL REPORTS about Dicumarol in 1941 by Meyer, Bingham, and Pohle (Madison), Butt, Allen, and Bollman (Rochester) aroused my interest, which was further whetted in October 1941, by Wright and Prandoni's lecture at the New York Academy of Medicine. These authors discussed the use of Dicumarol in venous thrombosis, pulmonary embolism, rheumatic heart disease, and peripheral arteriopathies. During 1942 at various times I talked about the possibility of utilizing Dicumarol in acute coronary thrombosis with myocardial infarction with Nelson Barker, Wilbur Duryee, Irving Wright, and others. Considerable doubt was expressed as to the likely results, but it was agreed that a clinical trial would be worthwhile. About this time, Clarence de la Chappelle noted an incidence of 12 per cent thromboembolism following acute infarction and stated, "Some day it may be demonstrated that [mural thrombi] will be prevented by the use of an anticoagulant such as heparin or dicoumarin." In June 1943, after hundreds of patients with thromboembolism had been treated with Dicumarol by various workers, I gave Dicumarol to my first patient with acute coronary thrombosis. After this initial venture, I continued to use Dicumarol routinely in acute myocardial infarction. No data on the use of Dicumarol in acute coronary thrombosis were available, but Barker had told me of its trial in a few cases, and after my study began, Wright and Duryee informed me that they had successfully used Dicumarol in 9 cases of coronary thrombosis. During 1944, casual references to the use of Dicumarol in acute coronary thrombosis appeared in papers describing its general use, Lam listing 3 cases, Evans 1 case, Oelter et al. 1 fatality, Townshend and Honingman 3 cases, and LeFevre mentioned 7 cases in 1945.

In October 1944 I reported the results of Dicumarol therapy in 30 patients with "acute coronary thrombosis" at a meeting of the Miami Heart Association (I keep these lantern slides for sentimental reasons). I was naive enough to believe, since the patients did well, the discussion would arouse interest in what seemed a truly constructive advance in the treatment of acute myocardial infarction, but instead of approbation, asides were audible anent my cerebral "slip" showing! In spite of the prevailing opinion that such therapy was meddling and dangerous, within another year I compiled the data obtained in 50 acute attacks, but this manuscript was returned with a frank note that it lacked scientific basis by the Journal of the American Medical Association. In my "President's Address," American Therapeutic Society, November 11, 1945, I related the results of my study. My closing remarks were, "Dicumarol is not the ideal therapy for coronary artery thrombosis, but it is a step forward. Two patients had each experienced three episodes of coronary thrombosis within two years, so were given Dicumarol continuously for 1 year as a protective measure, watching the prothrombin time at intervals. It may be more than coincidence they have not experienced a fourth attack. I look forward with confidence to the development of more easily controlled drugs to prevent intravascular thrombosis—or is this a fond dream of a middle-aging physician desirous of eluding the onslaught of time?" My data were buried in the Society Transactions and overlooked by other workers. The report was published* in full in January 1946. Because of similar studies by Wright, Peters, et al., the American Heart Association sponsored in 1946 an investigation of the value of anticoagulants in myocardial infarction. A debt of gratitude is owed to Irving Wright, Charles Marple, and D... ...y Beck ... hard work ... corr...

by 16 investigators. Parker and Barker reported good results in acute coronary thrombosis soon, and a flood of similar papers appeared. *In 1949* editorials appeared in several leading medical journals anent the use of anticoagulants in acute myocardial infarction. From June 1943 to June 1953 my associates and I treated 207 patients suffering acute myocardial infarction with anticoagulants. The over-all mortality rate was 14.3 per cent. (A 6 week interval was considered as the acute stage instead of the 4 week interval used by some authors.)

Twenty-two autopsies (71 per cent) were obtained Rupture of the ventricle was found in only 3 cases (1.3 per cent), which suggested that the size of the infarcted area was limited in extent by energetic anticoagulant therapy. In contrast, Waldron found 15 per cent of 71 cases treated with anticoagulants developed myocardial rupture and 4 9 per cent of 241 patients *not* treated with anticoagulants Mural thrombi were found in only 2 cases. We rarely encountered "pericarditis epistenocardica" in patients who were fully heparinized early.

Armand Quick's 1-stage method of prothrombin determination soon became established as a guide in adjusting the Dicumarol dosage. In 1943 Miami was not overflowing *with competent laboratory technicians so it* devolved on me to learn the pitfalls of prothrombin tests to make sure that technicians performed such tests accurately During this *early period I admitted a woman* with an acute attack to a hospital where the laboratory declined to set up a prothrombin method on the grounds it would seldom be used! Harrowing *experiences with prothrombin tests* done in outlying towns led to not a few acrimonious remarks about Dicumarol therapy, but eventually all hospitals and private laboratories in the area *instituted the needed coagulation* methods.

Most workers at first transposed the results of the prothrombin time to a hyperbolic serial dilution curve and *expressed results in* "percentage of prothrombin activity" rather than in "seconds of prothrombin time." Study of the pitfalls in computing "percentage" of prothrombin activity convinced me that maintaining the prothrombin time between 2 and 2½ times the normal would provide a satisfactory range of hypoprothrombinemia, and I urged an early Anticoagulant Panel of the American Heart Association to report prothrombin tests in "seconds" instead of "percentage." Shepard Shapiro stated in 1951, "The clinician should know the normal range of the thromboplastin used and the therapeutic range he wishes to establish in terms of time. With this knowledge the calculation of percentage is superflous; without it, the percentage figure is misleading." Some laboratories here and abroad computed a "percentage of prothrombin" or "clotting index" by a linear ratio of the normal prothrombin time compared to the patient's prothrombin time, which led to gross errors in adjusting Dicumarol therapy. The Link-Shapiro modification of Quick's method, comparison of the prothrombin time of a 12 5 per cent plasma dilution with that of whole plasma, has some advantages. Although the 2-stage prothrombin method is often advocated, in our hands it has not been of superior value in regulating anticoagulant dosage. In 1945, Hurn, Barker, and Magath pointed out that the nature of the thromboplastin used has great bearing on the validity of the prothrombin tests, and marked discrepancies arising from various thromboplastins employed were emphasized by Bramble. Knowledge about the vagaries of prothrombin tests has not become widely disseminated. Our laboratory ran thousands of tests between 1945 and 1955 using 2 different sources for thromboplastin (rabbit lung and brain) and the conflicting results with "pathologic" plasma were often so striking that repeated studies sometimes uncovered a hidden source of error, but more often the *variation* was due to the difference in the source of thromboplastins. Recently variable results obtained using different thromboplastins were carefully analyzed in an excellent *exposé* by Verstraete, Clark, and Wright.

When Moloney in 1948 showed the effect of Dicumarol on the clotting time in silicone-

treated tubes, I meticulously prepared such tubes, only to find that clotting was often delayed several hours, so I abandoned this procedure as impractical.

Heparin

Jay McLean, studying the blood clotting effect of cephalin, incidentally discovered the anticoagulant heparin in 1916; hence the finding of this natural anticoagulant as well as Dicumarol may properly be termed "serendipitous." McLean's recent obituary in the Journal of the American Medical Association made no mention of heparin, one part of which will prevent the clotting of *100,000 times its weight of blood! In 1936 heparin* was purified sufficiently by Charles and Scott to permit its clinical trial in thromboembolism. On the basis of animal experimentation, Best suggested in 1940 that heparin might prove *effective in the treatment of acute coronary* thrombosis, but it was seldom utilized during the next few years. Only after McLean's talk in 1945 I began to use it, and have relied on it more and more during the initial 7 to 10 days of the acute episode.

Helen Glueck and co-workers in Cincinnati reported on the combined use of heparin and Dicumarol in myocardial infarction in 1948. About the same time Loewe reported on the use of heparin* alone during the acute and healing stage of myocardial infarction. In 1949 I treated 24 patients with acute coronary thrombosis for 4 to 6 weeks with delayed action heparin† with good results except for occasional painful hematomas, but I failed to report my experience. Since the description by Stats and Newhof in 1947 of the advantages of concentrated heparin, I have used this preparation, preferably in concentration of 200 mg. per ml. The dosage ranges from 50 to 75 mg every 6 hours, given subcutaneously not intramuscularly, in the areolar tissue along the iliac crest. Heparin tolerance tests and heparin-retarded coagulation times as indicators of the need for heparin therapy proved unreliable. I found *no occasion to* use hyaluronidase with depot injections to minimize pain as suggested by Tuchman and Moolten. In spite of the fact that heparin induced bleeding sometimes, protamine sulfate was required to control bleeding in only 1 patient. *The prolongation of the prothrombin* time by heparin as first shown in 1946 by Long and Barker was sometimes a source of dosage error when changing from heparin to other anticoagulants, until we became more alert.

*Heparin in Pitkin Menstruum, Warner Company
†Depo heparin, Upjohn Company.

Long-Term Therapy in Coronary Disease

In February 1944 I began long-term anticoagulant therapy to forestall recurrent myocardial infarction, and made a preliminary report* before the Southern Medical Association in November 1946. Although Wright and Foley had begun continuous treatment in rheumatic heart disease with embolic episodes, *no trial of long-term anticoagulant therapy* to prevent myocardial infarction had been essayed. The first patient treated merits some description.

Patient J.R.T., aged 64 years, had an attack of posterior wall myocardial infarction in January 1943. Six months later, he had a severe anterior infarction and was treated with Dicumarol for 6 weeks. In February 1944, he developed a third attack. Dicumarol therapy was instituted again and this time was continued to see if additional attacks could be warded off. In December 1945, gross hematuria with renal colic appeared, but never recurred. In November 1946, hematemesis and tarry stools developed due to a bleeding peptic ulcer. Dicumarol was omitted for 5 weeks, then resumed because of worsening anginal pain. Attacks of bronchitis recurred frequently and pulmonary emphysema developed in 1948. The Dicumarol requirement was remarkably constant (700 to 800 mg weekly) until a summer holiday in 1948 in Nova Scotia when he imbibed ale instead of milk, a dietary change which reduced his Dicumarol requirement to 600 mg. weekly. He continued business activities up to 6 months before his death, when he gradually developed intractable congestive heart failure, azotemia, and anemia. As inanition increased, his Dicumarol requirement dropped to 500 mg. per week. He was comatose for 3 days before death. Continuous Dicumarol therapy had been followed for 90 months, except

*My associate, David Fassett, was co author

for 5 weeks during the bleeding ulcer episode. Autopsy showed no fresh coronary thrombus or infarction, old posterior wall infarction, calcified left ventricular aneurysm, nephrosclerosis, purulent bronchiolitis and emphysema, and healed gastric ulcer.

It would be an understatement to say it was an uphill battle, promoting the concept that permanent ambulatory anticoagulant therapy was feasible and worthwhile. In spite of the askant mien of my colleagues, I took heart in reading again a statement first made by E. V. Allen in 1945, "It is timely to consider that blood may normally clot in blood vessels *too well* to serve the interest of the health of man —*some time in the future there may be no* valid reason why the coagulability of the blood in man may not be maintained indefinitely and safely at a level which will not permit intravascular thrombosis." In 1949 Foley and Wright reported their results in 19 patients on long-term therapy, 5 of whom were "coronary" patients. Yet Bean in the same year stated that the use of anticoagulants in ambulatory patients as a prophylactic measure was out of the question! In 1950, Borg and I reported on 78 patients treated continuously to prevent recurrent myocardial infarction. Subsequent reports by Hellem, Keyes and co-workers, Suzman and co-workers, Owren, Coogan, and Davis, Tulloch and Wright, Manchester, and more recently Bjerkelund and Owren, indicated benefit from permanent anticoagulant therapy. A cooperative study comprising 1,091 cases treated by 10 physicians* for a total of 24,454 months, compiled by me in 1954, but only published recently, confirms the value of long-term anticoagulants in reducing the incidence of recurrent myocardial infarction and in lengthening the span of life after 1 or more attacks.

Impending Myocardial Infarction

During long-term anticoagulant therapy to prevent myocardial infarction, it appeared likely that some episodes of worsening anginal pain might well have ended in full-blown myocardial infarction had anticoagulants not been in force. This observation led me in 1946 to use anticoagulants in patients presumed to be showing premonitory signs of myocardial infarction, a condition which at the outset is indistinguishable from the clinical syndrome of acute coronary insufficiency since the eventual diagnosis is made only in retrospect after a number of days have elapsed. Results in 41 patients in this category constituted my "Address of Chairman," Section of Medicine, Southern Medical Association, November 1949, published in July 1950, and was the first paper on this topic in the world literature. Paul Wood had described the use of anticoagulants in 10 cases of "angina at rest" at the December 1948 meeting of the Medical Society of London. In April 1954 I reported results obtained in 150 additional cases at the American College of Physicians. My associates and I during the past 11 years have treated 313 private patients presenting premonitory signs of myocardial infarction, heparin being used for 1 week before instituting oral anticoagulants. Relief of pain was often strikingly coincident with full heparinization. Only 20 of the 313 patients (6.3 per cent) developed frank myocardial infarction, 5 of whom died within 30 days. Of the remaining 293 cases not developing frank infarction, none died within 60 days while using anticoagulants. Twenty-seven patients abandoned anticoagulants before the expiration of 60 days, of whom 16 (60 per cent) developed frank infarction during the ensuing 60 days. (Unpublished data except for an abstract in the program of the 1957 American Heart Association Clinical Sessions.) Similar observations have been reported since 1952 by Maynard, Thompson, Engelberg, Lenègre, and Beaumont, and in the past year by VanderVeer, Anderson, and Waaler.

Notes on the Risk of Hemorrhage

Clinical bleeding due to anticoagulants was first described by Prandoni and Wright in 1941, and by the year 1948, 28 deaths were recorded in the world literature ascribed to the use of antic

*Co-authors: John N. Keyes, Joseph F. Borg, Thomas J. Coogan, John J. Boehrer, William L. Mullins, Thornton Scott, Robert Page, George C. Griffith, Edward Massie.

obtained by *questionnaire further data from* 136 clinicians who reported that significant hemorrhage occurred in 2 per cent of 15,500 patients *with 35 deaths not previously reported*. My paper on "Risk of Hemorrhage" appeared in February 1950 and was later summarized as a guest editorial in the J.A.M.A. One instance of hemopericardium without myocardial rupture due to anticoagulant therapy in acute myocardial infarction was included, but the first case report dealing with this complication was made by Hammarsten in 1949 and was further emphasized by Goldstein and Wolff in 1951. Three deaths resulted primarily from performing lumbar sympathetic blocks when anticoagulant effect was in force, although hemorrhage was found elsewhere in these cases. The concomitant use of heparin and spinal anesthesia in 1 case, causing transverse hemorrhagic myelitis, and the performance of dorsolumbar sympathectomy without omission of anticoagulants in another, accounted for 2 deaths, both deaths exemplifying poor clinical judgment rather than faulty anticoagulation measures. Most instances of major bleeding were associated with pathologic lesions. The first case of vaginal bleeding in my experience occurred in an elderly woman being treated for coronary thrombosis who proved to have an early carcinoma of the cervix. Sometimes melena induced by anticoagulants led to the discovery of an occult lesion in the gastrointestinal tract.

An extraordinary hemorrhagic death* was that of a 53 year old man on long-term therapy to prevent recurrent infarction, who developed a peritonsillar abscess complicated by edema and hemorrhage into the cervical structures and larynx which produced fatal respiratory obstruction while he was en route to a hospital by ambulance. (Death might well have been prevented by earlier hospitalization.)

Although hematuria was the most common type of bleeding encountered, in no instance was it fatal or followed by added renal impairment. Ureteral colic from clots may follow the free use of vitamin K-1 in hematuria as first noted by me in 1945. One death due to hemorrhage from dissecting aortic aneurysm mistakenly treated as pulmonary infarction was reported by Evans in 1944 and I recorded 2 examples, one wrongly diagnosed as myocardial infarction, the other as saddle embolus. The incidence of fatal hemorrhage in the cooperative long-term study was 0.5 per cent in 1091 patients treated for an average of 22.4 months. Permanent use of anticoagulants is naturally associated with a greater risk of bleeding than when anticoagulants are used for a few weeks only.

In 1950 a patient to whom I had administered long-term Dicumarol therapy to prevent myocardial infarction for 6 months became psychotic and attempted suicide by slashing his wrists, so anticoagulants were stopped. He died a few years later with a recurrent infarction. A suicidal attempt by the self administration of sodium warfarin was recorded in 1952 by Holmes and Love.

In March 1954 I wrote:

> Most hemorrhagic episodes developed because of hypoprothrombinemia, but in some instances in patients using Cumopyran the prothrombin time was found well within the accepted safe therapeutic range, but the Lee-White coagulation time was abnormally prolonged, suggesting that in long-range anticoagulant therapy other coagulation factors may be upset.

In 1957 Herbert Sise and co-workers showed that phenylindanedione produces a deficiency of proconvertin factor (factor VII) and plasma thromboplastin component (PTC), thus accounting for some clinical hemorrhagic episodes in the absence of marked prothrombin deficiency. Currently an intensive study of these effects is going on in the Miami Heart Institute Anticoagulant Laboratory under the direction of Paul Boyles in patients on long-term therapy.

Side Effects of Anticoagulants

Alopecia as a toxic manifestation of dicoumarin anticoagulants seems more prevalent in Europe than in this country, and I have encountered only 2 examples, but alopecia induced by heparin is somewhat more frequent

*Related by Dr. R. V. Edwards.

Lack of toxic effects on the liver due to Dicumarol were reported by Meitus and Wasserman in 1953. Liver function studies and autopsy data from our patients on permanent therapy with oral anticoagulants revealed no hepatic injury.

One untoward effect of oral anticoagulants is the production of fatigue, malaise, or even anorexia in some patients Skin eruptions, occasionally scarletiniform, may occur. Febrile reactions are not common but one of my patients, whom I attempted to treat continuously, developed a fever in 1950 proven to be due to Dicumarol, and in the next year, during a trial of Tromexan, fever recurred and did not abate till the drug was stopped, but she was able to tolerate Hedulin. The same patient developed moderate alopecia. A colleague, Dr. Sidney Davidson, in West Palm Beach, had the reverse experience with a patient manifesting febrile reaction proved to be due to Hedulin but Dicumarol was well borne.

Heparin intravenously may induce shock-like reactions in "sensitive" subjects, as happened in one of my patients, and a few instances have followed its use intramuscularly Local reactions at the site of injection of heparin are usually not of consequence.